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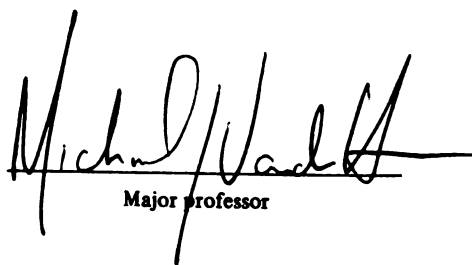
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**THE ASSOCIATION OF PREPARTUM
NON-ESTERIFIED FATTY ACIDS AND BODY CONDITION
WITH PERIPARTUM HEALTH PROBLEMS
ON 95 MICHIGAN DAIRY FARMS**

presented by

Paul Brian Dyk

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M.S. degree in **Animal Science**


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**THE ASSOCIATION OF PREPARTUM NON-ESTERIFIED FATTY ACIDS
AND BODY CONDITION WITH PERIPARTUM HEALTH PROBLEMS
ON 95 MICHIGAN DAIRY FARMS**

By

Paul Brian Dyk

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

MASTER OF SCIENCE

Department of Animal Science

1995

ABSTRACT

THE ASSOCIATION OF PREPARTUM NON-ESTERIFIED FATTY ACIDS AND BODY CONDITION WITH PERIPARTUM HEALTH PROBLEMS ON 95 MICHIGAN DAIRY FARMS

By

Paul Brian Dyk

Ninety-five dairy farms above Michigan DHIA average of 8760 kg of milk/cow/year were visited four times within a 6 week period. At each visit, a body condition score (BCS) and a blood sample were taken from each Holstein animal that was within 35 days of the expected date of parturition. Plasma from the blood sample was analyzed for non-esterified fatty acids (NEFA). Higher prepartum NEFA concentrations were associated with a higher incidence of dystocia, retained placenta, ketosis, displaced abomasum, and mastitis but not milk fever. Animals with higher BCS scores had a higher incidence of ketosis and displaced abomasum but not dystocia, retained placenta, milk fever, or mastitis. Prepartum NEFA concentrations were elevated in animals with higher BCS, and lower predicted transmitting ability for milk. BCS were lower in animals that had higher predicted transmitting ability for milk. Decreasing prepartum lipid mobilization may result in fewer peripartum health problems.

To God, the Creator and Sustainer of All.

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INTRODUCTION

The time around calving is a critical period in the life of a dairy cow. The initiation of lactation, the stress of calving, and a high incidence of health problems make it a key transition phase in the life of a dairy cow. There is great economic incentive to make this transition period smooth and free of problems. Currently, peripartum health problems result in losses of ~\$50 million/year in Michigan due to increased veterinary costs and loss of milk production (Ferris and Fogwell, 1984).

A challenge faced by the dairy animal in the last couple of weeks prepartum is maintaining a positive nutrient balance. Poor nutrient balance may result from a combination of decreased feed intake in the last couple weeks prepartum and increased energy requirements due to lactogenesis and increasing fetal needs.

Prepartum non-esterified fatty acids (NEFA) were used in this study as an indicator of nutrient balance. When a cow consumes less energy than required, body fat is mobilized and concentration of plasma NEFA increases. Many of these NEFA are taken up by the liver and reesterified to TG. Excess TG accumulation results in fatty liver, which may cause more health problems such as ketosis and displaced abomasum after calving. In addition, poor nutrient balance as indicated by elevated NEFA may suppress the immune system and thus lead to more health problems such as mastitis.

Body condition score, which is a measure of body fatness in the dairy animal, was also examined in this study. High BCS has been reported to reduce feed intake and increase the incidence of health problems such as ketosis and mastitis.

The hypothesis of this study is that elevated concentrations of NEFA in plasma and higher BCS before calving will increase the incidence of peripartum health problems in dairy cattle.

Our specific objectives were:

- 1) To determine if elevated prepartum NEFA concentrations are associated with more health problems in the first 60 days after calving.
- 2) To determine if higher BCS is associated with more health problems in the first 60 days after calving.
- 3) To determine what factors affect NEFA concentration and BCS in dairy cattle before calving.

The specific health problems examined were: dystocia, retained placenta, milk fever, ketosis, displaced abomasum, and mastitis.

LITERATURE REVIEW

Relationship of parity and peripartum health problems

There is a significant relationship between disease and parity. As shown already in 1923 (Sjollema and Van Der Zande), and again in 1956 (Shaw), the incidence of ketosis increases as parity increases. In Swedish Red and White and Swedish Friesian cattle, there was an increase in retained placenta, ketosis, and mastitis as parity increased (Emanuelson et al., 1993). In another Scandinavian study with Finnish Ayrshire cattle, the incidence of dystocia, milk fever, and ketosis was greater during the second lactation than the first lactation (Mantysaari et al., 1991).

Relationship of BCS and peripartum health problems

Body condition score (BCS) is a measure of subcutaneous lipid stores based on a visual appraisal (Wildman et al., 1982). Domecq et al. (1994) showed that BCS was positively correlated (ranging from .49 to .73) to ultrasound measurements of subcutaneous fat at the lumbar, thurl, and tailhead regions. Otto et al. (1991) did a carcass study to evaluate the correlation of BCS and fat content of the carcass. After slaughter, 9th to 11th rib sections were analyzed for ether extract content. Each unit

increase of body condition score was associated with a 12.7% increase in ether extract. Waltner et al. (1994) slaughtered 23 animals and showed that the percent of empty body fat (does not include fat from internal organs) could be accurately estimated ($r^2=.78$) from body weight and body condition score. Based on these ultrasound and carcass studies, BCS is reliable as a relative indicator of body fatness in dairy cattle.

The link between overconditioned animals and disease has been quoted by some individuals; however, the evidence is not conclusive. In an often referenced paper, Fronk et al. (1980) conclude that the overconditioned animals in their study had more health problems; however, there is no statistical analysis to back this claim. In addition, there are no clear descriptions of diseases and there is no indication as to whether cases of a disease were counted twice if they occurred in one animal. Fronk et al. conclude that there are more cases of ketosis, milk fever and mastitis in overconditioned animals but the limited number of cows (44 animals) in this study make it difficult to extrapolate this to the Holstein population. A review by Morrow (1975) on "Fat cow syndrome" says that overconditioned animals have a higher incidence of milk fever, ketosis, displaced abomasum, indigestion, mastitis, etc.; however, the paper again does not give evidence to support this claim. Although these two papers are often cited as the basis for the idea that overconditioned animals have more health problems, they clearly should not be. More recently, Ruegg (1995) showed that higher BCS at calving was not associated with disease in 429 animals in 13 herds. Gearhart et al. (1990) reported that overconditioned animals (≥ 4 on a scale of 1 to 5) did not have a higher incidence of ketosis or displaced abomasum in 561 animals.

Despite a lack of evidence of a direct link between BCS and disease, the mechanism for such a link may be through the effect of BCS on DMI and thus on energy balance. Some investigators have shown lower DMI in overconditioned animals (Garnsworthy and Topps, 1982; Treacher et al., 1986) and concluded that this may result in more health problems; however, Holter et al. (1990) did not see lower DMI in overconditioned animals. Grummer sums up the effect of BCS on intake quite well in his 1993 review of ruminant lipid metabolism: "The importance of body condition as a determinant of prepartum feed intake has not been examined critically."

Relationship of NEFA and peripartum health problems

An important indicator of whether or not a cow will develop a peripartum health problem may be the concentration of plasma non-esterified fatty acids (NEFA) in the prepartum period. The concentration of plasma NEFA is proportional to the rate of lipid mobilization in the cow, and lipid mobilization is proportional to the shortfall of dietary energy to meet an animal's requirements (Mills et al., 1986). Plasma NEFA are taken up by the liver proportional to their concentration in plasma and the rate of blood flow to the liver (Bell, 1980). Because the ruminant liver does not export very low density lipoproteins (VLDL) in significant quantities, uptake of NEFA may lead to fatty liver. Fatty liver has been connected to ketosis which may indirectly lead to other health problems such as displaced abomasum (Curtis et al., 1985). However, fatty liver may not be the only mechanism whereby elevated NEFA could be associated with more health

problems. A higher concentration of plasma NEFA is an indication of negative energy balance or poor nutrient balance, which may cause a general suppression of the immune system and consequently lead to more health problems such as mastitis.

Plasma NEFA concentrations around parturition

Prepartum plasma NEFA are the focus of my study, not postpartum plasma NEFA, because prepartum plasma NEFA are more likely to cause fatty liver. In the prepartum period, some plasma NEFA will be used by tissues as an energy source while the rest will be taken up by the liver. Thus, if plasma NEFA concentrations are high, triglycerides will accumulate in the liver and eventually result in “fatty liver”. After calving, however, if plasma NEFA concentrations are high, the mammary gland will remove much of the plasma NEFA for use in milk synthesis. This is supported by reports that the liver is already infiltrated by TG at the time of calving (Grummer et al., 1993; Gerloff et al., 1986).

Immediately around the time of calving (+/- 24 hrs), plasma NEFA may increase from 300 μ M to over 900 μ M (Bertics et al., 1992; Studer et al., 1993). Four possible causes for this increase are: 1) a decreased intake of energy, 2) an increased energy need by the animal or fetus, 3) hormonal shifts related to lactogenesis and calving, or 4) a combination of these possibilities. Although DMI drops over the last 10 days prepartum, the drop 1 day before calving likely is not the cause of high NEFA at parturition, because the NEFA surge occurred even when cows were force fed through a rumen cannula to

maintain DMI (Bertics et al., 1992). The possibility that the surge of plasma NEFA is caused by a sudden need for more energy associated with lactogenesis seems more likely, but there is little evidence in the literature to support or refute this hypothesis. Perhaps the most likely cause of the NEFA surge at calving may be the increase in plasma epinephrine or norepinephrine occurring around parturition (Grummer, 1993). Finally, the increase in NEFA at calving is probably caused by a combination of these factors. However, the possibility that this large increase in NEFA on the day before calving could by itself cause fatty liver seems unlikely.

In addition to the NEFA surge at calving, a moderate increase in NEFA occurs over the last week or two before calving (Grummer, 1993). And in fact, this moderate increase over the last week seems more important than the surge at calving. Drenching animals with propylene glycol, a glucose precursor, once daily from about 10 days prepartum until parturition significantly lowered plasma NEFA concentrations (234 μ M vs 403 μ M) from 6 to 1 days prepartum compared to those of control animals (Studer et al., 1993). However, NEFA increased similarly on the day before calving in both drenched and control animals, and DMI decreased similarly in both groups beginning 10 days before calving. Importantly however, drenched animals had 32% less TG in liver at 1 day after calving. Thus, the apparent decrease in the rate of lipolysis as indicated by plasma NEFA for 1 week prepartum resulted in a decrease in liver TG concentration. This indicates that the NEFA surge immediately around calving does not account for all the TG infiltration and subsequent problems. Precisely how many days before calving plasma NEFA concentration may be important has not been determined.

Causes of NEFA elevation during the last week prepartum

The increase in NEFA concentration during the last week before calving may be caused by the general decrease in DMI observed over the last 2 weeks (Figure 1). In a study by Bertics et al. (1992), animals were force fed through a rumen cannula to maintain DMI prior to calving. In control animals, liver TG increased 227% in last 17 days of gestation; however, the increase was only 75% in force-fed animals, which suggests that maintaining DMI reduces TG infiltration of the liver but does not eliminate it. No differences in plasma NEFA were observed between the two groups but authors point out that there was a tremendous amount of variation between cows so that they were unable to statistically detect a difference. In both groups, NEFA rose exponentially within a day of calving.

The cause of the drop in DMI around calving is unclear but may be related to space in the abdominal cavity, hormonal shifts occurring around calving, or the stresses of calving and lactogenesis and the management changes associated with them.

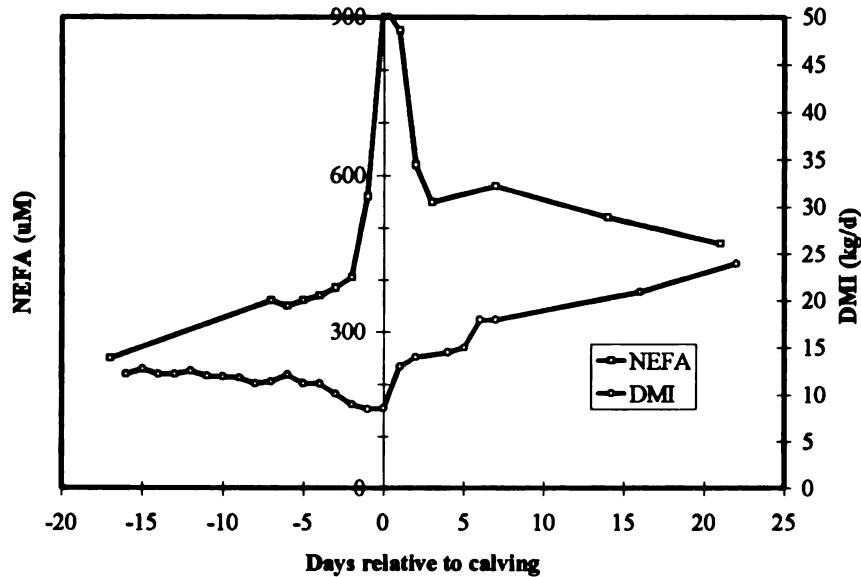


Figure 1. Periparturient DMI and plasma NEFA concentrations of multiparous cows (adapted from Grummer, 1993)

Another possible reason for the increase in NEFA around calving may be the increased needs of the animal and fetus. As the fetus continues to grow throughout the dry period, more energy may be needed for its maintenance and development. In addition, as the mammary gland prepares for the upcoming lactation in the last week of gestation, there may be an increased need for energy. Currently the National Research Council (NRC) does not account for these possible changes in energy requirements in their recommendations for the dry cow (NRC, 1989).

Plasma NEFA through Fatty Liver as a cause of health problems

A fatty liver is one that has become infiltrated with triglycerides. The liver may take up a minor amount of TG from chylomicra in blood. In sheep, 10% of the plasma

pool of chylomicra is removed by the liver and 20% is removed in dogs (Bruss, 1993). Thus, in a dry cow fed a typical diet (<5% fat), chylomicra likely are a minor source of liver TG.

Because chylomicra are only a minor source of liver TG, a fatty liver is caused by an increase of TG that come from the reesterification of NEFA. There is very little fatty acid synthesis within the ruminant liver, therefore the ruminant TG are formed from plasma NEFA. Uptake of plasma NEFA by the liver is proportional to the NEFA concentration in the blood (Bell et al., 1980) with a removal rate of 7-25% of the NEFA presented to the liver (Emery et al., 1992). NEFA in the liver can be completely oxidized to CO₂, incompletely oxidized to ketone bodies, or reesterified to TG (Bruss, 1993). Because the ruminant liver does not export VLDL in appreciable amounts, the balance of NEFA uptake and NEFA oxidation in the liver determines the degree of TG accumulation in the liver. This leads to the conclusion that NEFA in plasma is the best indicator of the accumulation of liver fat (Reid and Roberts, 1983; Roberts et al., 1981).

The mechanism whereby fatty liver might affect peripartum health is not clear, except in the case of ketosis, because an increase in liver lipids is considered part of the etiology of ketosis (Baird, 1982; Littledike et al., 1981). The best evidence for a link between ketosis and fatty liver is the work conducted at Iowa State University using an induced ketosis model (Veenhuizen et al., 1991). To induce ketosis, animals were fed at restricted intake and fed 1,3-butanediol as a source of ketone bodies. Induction of ketosis began 15 days postpartum and clinical ketosis occurred by 45 days postpartum. In animals with induced ketosis, liver triglycerides on a wet weight basis increased from 2%

at 5 days after calving, to 10% at 14 days before ketosis occurred. The idea that fatty liver precedes ketosis is supported by findings that showed animals that developed ketosis during lactation already had a fatty liver at 1 day after calving (Grummer, 1993; Baird, 1982).

One possible means by which fatty liver may cause ketosis is through impaired liver function (Mills et al., 1986). Impaired liver function is indicated by mitochondria that seem malformed and cristae that are disordered and less distinct in the cells of a fatty liver (Reid and Collins, 1980). Increased fat also decreases diffusion of cell metabolites through the cell (Bruss, 1993), decreases the activity of gluconeogenic enzymes (Mills et al., 1986), and decreases liver glycogen concentrations (Young et al., 1990). Perhaps these changes in glucose metabolism are key in the etiology of ketosis.

Other possible connections of NEFA to health problems

Displaced abomasum may occur more frequently in animals that have ketosis (Curtis et al., 1985; Markusfeld, 1986), but whether ketosis causes displaced abomasum or if displaced abomasum causes ketosis, or both are caused by some other phenomenon is not known. When ketotic animals reduce their intake, gut motility may be decreased, which could result in displaced abomasum. On the other hand, an animal with a displaced abomasum also would reduce intake and consequently might then become ketotic. In any case, it seems likely that if prepartum NEFA are elevated in animals that have ketosis, then animals that have displaced abomasum will also have higher prepartum NEFA.

Elevated prepartum plasma NEFA may also be related to a high incidence of some peripartum health problems because high NEFA indicate that an animal is in poor nutrient balance. Animals that are in poor nutrient balance may have a suppressed immune system (Rhoads, 1980; Keusch, 1981; Dreizen, 1979; Gross and Newberne, 1980). The immune system is suppressed around parturition (Weigel et al., 1992), but whether nutrition plays a role in this suppression is not known. Plasma cortisol which is elevated around parturition (Horst and Jorgensen, 1982) and suppresses immune function (Burton et al., 1995; Bateman et al., 1989; Roth, 1983), is increased during long-term malnutrition (Dwyer and Stickland, 1992). If an animal is under stress as in the case of malnutrition, perhaps the associated immunosuppression is a result of elevated cortisol (Elvinger et al., 1992).

An animal with a suppressed immune system is more susceptible to mastitis (Weigel et al., 1992). In addition, a suppressed immune system may cause a higher incidence of retained placenta (Cai et al., 1994 and Gunnink, 1984). The calf and the associated fetal membranes are immunologically foreign to the animal, and suppression of the immune system during pregnancy prevents the rejection of the calf (Billingham and Beer, 1984; Jacoby et al., 1984). However after calving, the placenta and fetal membranes must be rejected. If the immune system is suppressed at the time of calving, proper immunological rejection and expulsion of these foreign tissues may not occur. If these tissues are not expelled within 24 hours after calving, a case of retained placenta is said to have occurred. If poor nutrient balance causes immunosuppression, then a positive correlation of plasma NEFA and the incidence of mastitis and retained placenta might be expected.

MATERIALS AND METHODS

Farm selection

On June 19, 1993, letters were sent to over 300 farms across Michigan to solicit their involvement in a dry cow study. Letters were only sent to farmers that were enrolled in the Dairy Herd Improvement Association (DHIA) and had a current herd average above the Michigan DHIA average of 8760 kg (19300 lb.) of milk per cow per year. The farmers were asked to fill out and return a questionnaire (Appendix A, Figure A.1.) if they wanted to participate in the study. Of the over 300 letters sent out, 118 questionnaires were returned. Of these, 104 farms were visited.

Farm visits

The 104 farms were divided between the investigators Roy Emery (42), Paul Dyk (32), Herb Bucholtz (23), and Richard Cameron (7). Each investigator was responsible for collecting and recording information on the assigned farms. Each farm was visited 4 times within a 6 week period with a minimum of 6 days between each visit. The farm visits occurred between October 27, 1993, and January 25, 1995.

At the first visit, the investigator recorded management and nutrition information on standardized sheets (Appendix B, Figure B.1.). However, for farms (73 of the 104 farms) visited for the first time after May 1, 1994, an improved version of this sheet which was more specific was used (Appendix B, Figure B.2.).

At the first visit, all Holstein cows and heifers that were within 5 weeks of calving were identified and restrained. At some farms, identification or restraint of some animals, usually heifers, was not possible; these animals were excluded from the trial. A body condition score (BCS) was determined on a scale of 1 to 5, with 5 being very fat and 1 being very thin (Wildman et al., 1982). Prior to the initiation of the trial, investigators met to standardize their body scoring technique using a chart from Elanco Products Company (Indianapolis, IN, USA). In addition to BCS, blood was sampled via a tail vessel. Samples were put into ice within 15 minutes, brought back to the lab, and centrifuged at 3000 rpm for 15 minutes. Plasma was siphoned off and put into labeled plastic tubes and stored at -20 °C.

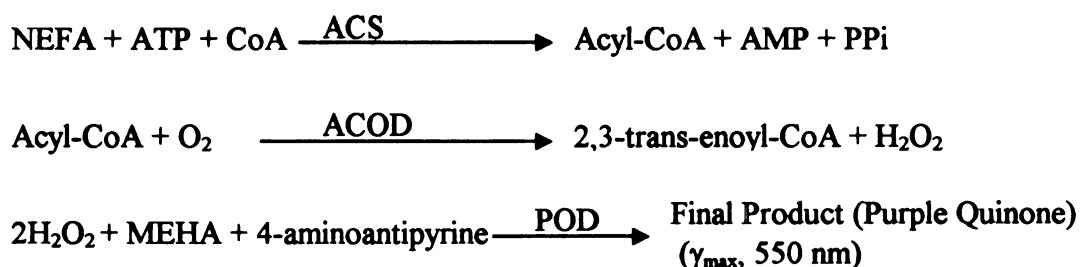
At the conclusion of the first visit the farmers were given sheets to record health information on animals involved in the study. These sheets were also revised (after the first 31 farms) to improve recording of dates for diseases (compare Appendix C, Figures C.1. and C.2.).

At the second, third, and fourth visit, BCS was evaluated and blood was sampled from animals within 5 weeks of expected date of calving. The animals on the second, third and fourth visit included animals from the previous visit that had not calved, and any other animals that were within 5 weeks of expected date of calving. Changes in management or nutrition were also recorded.

Lab method for NEFA analysis

Samples were analyzed for NEFA using a commercially available kit (NEFA-C kit, Wako Chemicals USA, Richmond, VA) with modifications by McCutcheon and Bauman

(1986), Sechen et al. (1990), and Johnson and Peters (1993). The kit was an enzymatic, colorimetric test, where NEFA in the presence of adenosine triphosphate (ATP), coenzyme A (CoA) and acyl-CoA synthetase (ACS) forms Acyl-CoA and the by-products adenosine monophosphate (AMP) and pyrophosphate (PPi). Acyl-CoA is then oxidized by acyl-CoA oxidase (ACOD) to produce 2,3-trans-enoyl-CoA and hydrogen peroxide (H_2O_2). H_2O_2 is combined with 3-methyl-N-ethyl-N-(β -hydroxyethyl)-aniline (MEHA), 4-aminoantipyrine and peroxidase (POD) to give the final purple product which is a quinone. This final product can be measured colorimetrically at 550 nm. The chemical basis is represented by the following reactions:



The NEFA kit included the following:

- 1) 6 vials of Color Reagent A (CRA) - ACS (3 U/vial), AOD (30 U/vial), CoA (7 mg/vial), ATP (30 mg/vial), 4-aminoantipyrine (3 mg/vial)
- 2) 1 bottle of CRA diluent - phosphate buffer (pH 6.9, 50mM), magnesium chloride (3000 μM), surfactant, stabilizer
- 3) 6 vials of Color Reagent B (CRB) - ACOD (132 U/vial), POD (150 U/vial)
- 4) 1 bottle of CRB diluent - MEHA (1200 μM), surfactant
- 5) NEFA Standard - Oleic acid (1000 μM), surfactant, stabilizers

Each vial of Color Reagent A was diluted with 10 mL of Color Reagent A diluent and 13.3 mL of phosphate buffer (50 mM, pH 6.9) and stored at 4°C. Each vial of Color Reagent B was diluted with 20 mL of Color Reagent B diluent and 33.3 mL of phosphate buffer and stored at 4°C. Working standards (1000, 500, 250 and 0 μ M) were prepared by diluting the NEFA standard provided in the kit with phosphate buffer.

The NEFA analysis used 96-well, flat-bottomed, polystyrene microtiter plates (Corning Glass Works, Corning, NY). Five μ L of plasma or standard was pipetted into the wells using a positive displacement pipetter. Figure 2 shows the layout of each plate in the analysis. The left side (A1 to H1) was used for the working standards while the bottom right cells (C12 to H12) contained internal standards of the analysis. These internal standards represented one animal that had low plasma NEFA (Cow A or Cow C) and one animal that had high plasma NEFA (Cow B or Cow D). The internal standards were used as a check on intra-assay and inter-assay variation; the values from the internal standards were not used to adjust NEFA concentrations. All standards were pipetted after all the plasma samples had been pipetted. Plasma samples were pipetted into the plate in duplicate starting at well A2, proceeding to H2, then A3 to H3, A4 to H4, etc. Plates were placed in 4°C while other plates were being pipetted.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	Sample 1 Repl. 1										Sample 41 Repl. 1
B	1000	Sample 1 Repl. 2										Sample 41 Repl. 2
C	500	Sample 2 Repl. 1										Cow A or Cow C
D	500	Sample 2 Repl. 2										Cow A or Cow C
E	250	Sample 3 Repl. 1										Cow A or Cow C
F	250	Sample 3 Repl. 2										Cow B or Cow D
G	0	Sample 4 Repl. 1										Cow B or Cow D
H	0	Sample 4 Repl. 2										Cow B or Cow D

Figure 2. Layout of microtiter plate for each plasma NEFA assay. Working standards were in Column 1, internal standards were in wells C12 to H12 and samples were in remaining wells.

Before each assay, specific farms were chosen and all samples from each farm were removed from the freezer and thawed at 4°C overnight or under cold tap water (15°C). Samples were kept at 4°C during the pipetting. Two data sheets were generated for the assay. One sheet (see example in Appendix D, Figure D.1.) was identical to layout of the plate and contained all the information for each sample for each well. This information was identical to the information on the label of the plasma sample. The other sheet (Appendix D, Figure D.2.) was placed underneath the plate during the pipetting and only had the cow number for each well. This procedure was used to ensure that the proper sample was placed in the appropriate well.

After all samples were pipetted, 100 µL of the Color Reagent A was added to each well. The plate was then shaken in the microtiter plate reader and placed at room temperature. After 30 minutes, 200 µL Color Reagent B was added to each well, and the plate again was shaken in the microtiter plate reader (Biolog Microstation, Biolog Inc., Hayward, CA) and placed at room temperature. After 30 minutes, the absorbence of light at 550 nm for each sample was measured in the microtiter plate reader. A regression equation was set up using the working standards from all the plates in the assay from that day. From this equation, light absorbence of the plasma samples were used to estimate NEFA concentrations. Any plasma samples that had replicates with a coefficient of variation greater than 20% were reanalyzed.

Some blood samples had hemolyzed because of the extreme cold conditions in the winter of 1993-94. The ice chests that held the samples at the farms became so cold that

the blood samples froze. The NEFA of these samples were analyzed and adjusted using procedures outlined by the NEFA kit.

Raw data editing and summarization

Information from the farms and NEFA analysis was entered into Microsoft Foxpro 2.6a for Windows[®]. Data were entered into three databases in Foxpro. One database was for the health and DHIA information for individual cows; the database was set up like the health sheets for ease of data entry. A second database had descriptive parameters of the farm such as date of visits, DHIA farm numbers etc. The third database contained information pertinent to the date of the visit such as BCS, NEFA, date of lab analysis etc.

The health problems that were monitored in this study were: twins, dystocia, retained placenta, milk fever, ketosis, displaced abomasum, and mastitis. Health problems were defined in the following way:

a) an animal was considered to have had twins, a retained placenta, milk fever, ketosis, or a displaced abomasum if it was reported on the health sheet the farmer filled out (Appendix C). Whether or not the farmer reported when the disease occurred did not matter.

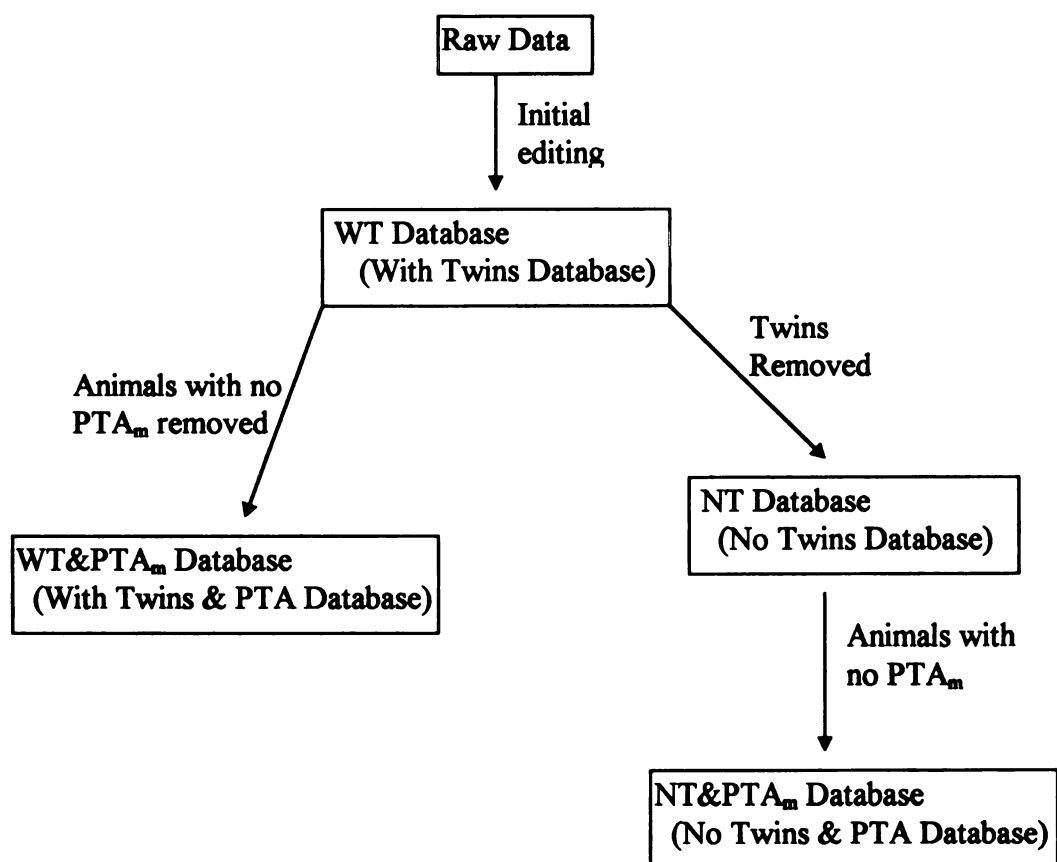
b) an animal was considered to have had dystocia if the farmer reported a 4 or 5 in the calving difficulty column of the health sheet (Appendix C). A 4 was designated as a hard pull and a 5 was designated as surgery.

c) an animal was considered to have had peripartum mastitis if the farmer reported that the animal had mastitis within the first 10 days. If the farmer did not report when a case of mastitis occurred, the mastitis case was ignored.

d) an animal was considered to have general mastitis if the farmer reported that the animal had mastitis within the first 60 days. If the farmer did not report when a case of mastitis occurred, the mastitis case was ignored.

The Four Databases used for statistical analysis

For the statistical analysis there were four sets of data that were used. The raw data were edited to develop a general database of all animals. Animals that had twins were removed to generate another database. From these two databases, the other two databases were derived by removing animals that did not have PTA_m value (predicted transmitting ability for milk). The relationships of the databases can be summarized as follows:



WT Database

This database included all animals with and without twins. This database will be designated as the “WT Database” which stands for **With Twins Database**, To arrive at the WT database, the following groups of animals were removed from the raw database:

- a) animals without a reported calving date
- b) animals without a plasma sample between -14 and 0 days prepartum
- c) animals that did not have a DHIA animal identification number
- d) animals that were not the following ages at calving (Ngwerume, 1994):
 - 1) between 18 and 36 months for first parity animals
 - 2) between 30 and 48 months for second parity animals
 - 3) greater than 42 months for third or greater parity animals
- e) any animals that had identical DHIA cow numbers

WT&PTA_m Database

The next database was formed by using the WT Database and removing any animals that did not have a calculated or parent average for PTA_m. This database, made up of only animals with valid PTA_m values, will be designated as “WT&PTA_m Database” which stands for **With Twins & PTA_m Database**. The calculated and parent averages of PTA_m were obtained from the last DHIA records before 100 days postpartum. Parent averages were not calculated in cases where the dam and/or sire were missing a PTA_m value. An additional 1095 lb. was added to the PTA_m values that were reported after January 4, 1995, because of the USDA genetic base change (from New and Improved Genetic Evaluations January 1995, Pamphlet of National Association of Animal Breeders

P. O. Box 1033, Columbia, Missouri, 65205-1033, USA). All animals that did not have a calculated or parent average for PTA_m greater than -174 lb. (20 animals) were removed because they represented outlying animals that could not be incorporated into the statistical analysis. The WT Database and WT& PTA_m Database were only used for analyses studying twins directly.

NT Database

For all other statistical analyses, animals that had twins were excluded. This led to the third database where animals bearing twins were removed from the WT Database. This database was designated as the “NT Database” which stands for No Twins Database. The objective of the study was to determine nutritional and managerial factors in the peripartum period that would affect the incidence of health problems. Animals bearing twins would have skewed results and led to incorrect conclusions because they had much higher NEFA in plasma and lower BCS.

NT& PTA_m Database

For the final database, animals that did not have valid PTA_m values were removed from the NT Database. This fourth database was designated “NT& PTA_m Database” which stands for No Twins & PTA_m Database. The criteria for the PTA_m values were the same as in the development of the WT& PTA_m database.

Statistical Analysis

Disease analyses using Cochran-Mantel-Haenszel Statistics

Cochran-Mantel-Haenszel (CMH) statistics in Statistical Analysis System (SAS, 1991) were used to study the relationship of parity, NEFA concentration, BCS, and PTA_m to health problems because health data was categorical. However, because CMH statistics make use only of categorical data, it was necessary to categorize any continuous data. Parity was a) 1, b) 2, c) 3 and greater as derived from DHIA records. The BCS from each animal over the last 2 weeks prepartum was averaged and grouped in the following way:

- a) Very Low BCS Group - $BCS \leq 2.75$
- b) Low BCS Group - $BCS > 2.75$ and ≤ 3.25
- c) Medium BCS Group - $BCS > 3.25$ and $BCS < 4$
- d) High BCS Group 4 - $BCS \geq 4$

For all analyses with PTA_m , the following PTA_m Groups were used:

- a) Low PTA_m Group = $PTA_m \geq -174$ lb. and < 826 lb.,
- b) Medium PTA_m Group = $PTA_m \geq 826$ lb. and < 1826 lb., and
- c) High PTA_m Group = $PTA_m \geq 1826$ lb..

NEFA values could not be categorized the same way because NEFA values were strongly affected by days prepartum. To compare NEFA values, each plasma NEFA value for each animal was expressed as a percentage of the average NEFA for all animals from the same day prepartum (Figure 3). If an animal had two samples from the last 2 weeks, the average of these percentages was used as the value for that animal. Three NEFA groups were then formed:

- a) Low NEFA Group - cows with average NEFA percent below 75.
- b) Medium NEFA Group - cows with average NEFA percent between 75 and 125.
- c) High NEFA Group - cows with average NEFA percent above 125.

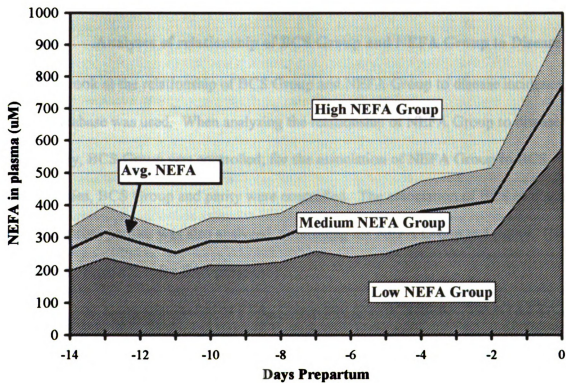


Figure 3. Plasma NEFA in NT Database. Each sample from each cow was expressed as percent of the average of all animals on the same day prepartum.

Analysis of relationship of Parity to Disease

Using the NT Database, the relationship of parity to disease was studied with CMH statistics in SAS. NEFA Group and BCS Group were controlled in one analysis and not controlled in the subsequent analysis.

Analyses of relationship of BCS Group and NEFA Group to Disease

To look at the relationship of BCS Group and NEFA Group to disease incidence, the NT Database was used. When analyzing the relationship of NEFA Group to disease within parity, BCS Group was controlled; for the association of NEFA Group to BCS across parities, BCS Group and parity were controlled. The association of BCS to disease incidence across parities was also analyzed, controlling for NEFA Group and parity. (See Appendix E, Figure E.1. for SAS program.)

To look at the relationship of PTA_m Group and disease incidence, the NT&PTA_m Database was used. When analyzing the relationship of PTA_m to disease, NEFA Group, BCS Group, and parity were controlled; the relationship was also analyzed controlling only for NEFA and BCS Group. Finally the relationship of PTA_m and disease incidence was analyzed, controlling for no other factors.

Analysis of relationships of NEFA, BCS, PTA_m, Herd, and Parity

To look at the effect of BCS group, herd and parity on NEFA, the NT Database was analyzed using the GLM (General Linear Models) option in SAS. Week -1 and week -2 prepartum were analyzed separately. The concentration of plasma NEFA was used as the dependent variable, and BCS Group, herd, and parity as independent variables. Day

prepartum was used as a covariate because of the sharp rise in plasma NEFA at calving.

To look at the effect of PTA_m Group on NEFA, the same model was used except PTA_m Group was used as additional independent variable, and the NT&PTA Database was used.

To look at the effect of parity and herd on BCS, the GLM option in SAS was used with the NT Database. BCS was used as a dependent variable with herd and parity as independent variables. The effect of PTA_m Group on BCS was studied using the NT&PTA Database and the same model except PTA_m was put in as an additional independent factor.

Analysis of twinning

To look at the effect of twins on NEFA and BCS, the WT Database was used. When the effect of twins on NEFA was analyzed the GLM procedure in SAS was used. NEFA was the dependent variable, and BCS Group, herd, parity, and twins were put in as independent variables. When the effect of twins on BCS was analyzed, BCS was used as the dependent variable, and parity, twins, and herd were independent variables. To look at the relationship of PTA_m and twins, the CMH option in SAS was used on the WT&PTA Database. Finally, to determine if disease incidence was higher in animals with twins, the WT Database was analyzed using the Chi-Square analysis of SAS.

RESULTS

Database summary

Of the 104 farms visited, only 95 farms were used in the data analyses for this thesis. The other farms were not used because health sheets were not returned or because no animals fit the criteria for our analysis. The raw health database contained 2577 animals. Of these, 26 did not have calving dates, 192 did not have any blood samples, 119 could not be linked to a DHIA cow identification number, 26 animals did not meet parity criteria, and 19 had insufficient or incorrect DHIA information, such as multiple animals with identical DHIA numbers. Of the remaining 2195 animals, 1655 had at least one blood sample in the last 2 weeks (day -14 to day -1) prior to parturition. These 1655 cows were the base of the analyses (WT Database) for this thesis. Ninety-nine of these animals had twins and were not used for analyses involving single births (NT Database). Of the remaining 1556 animals, 1093 ($\approx 70\%$) had valid PTA_m values (WT& PTA_m Database).

Prepartum NEFA concentrations in plasma

In analyzing the raw data, plasma NEFA concentration did not vary until about 2 weeks before calving (Figure 4). From -14 to -4 days prepartum, plasma NEFA concentration increased from 300 μM to 400 μM . Then from day -4 to day 0, plasma

NEFA concentration doubled to 800 μM . Plasma NEFA concentration was lowest for parity 2 animals but increased significantly in the last days before calving for all parities (Figure 5).

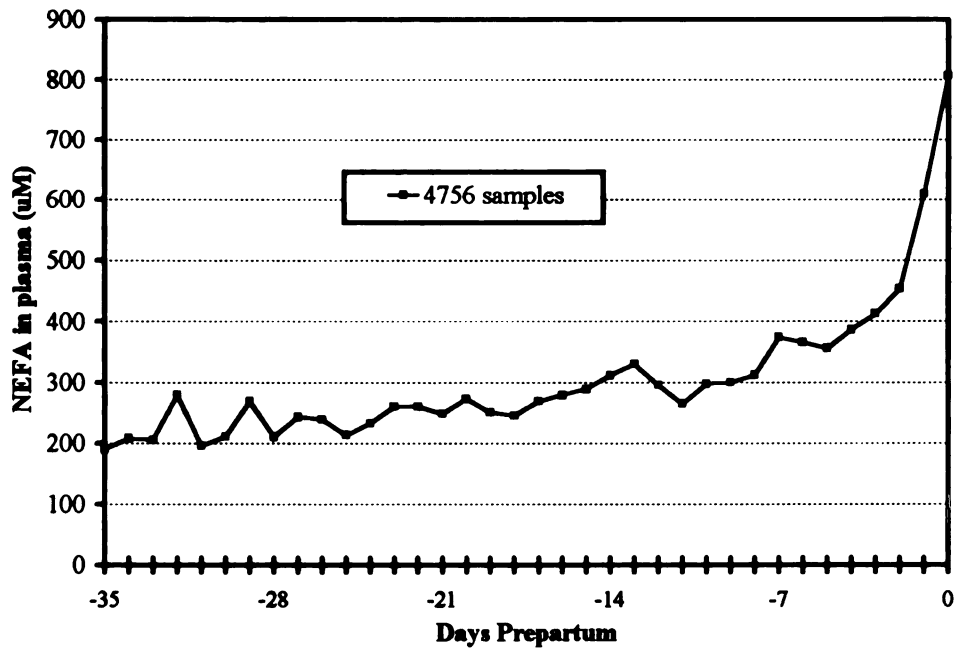


Figure 4. Plasma NEFA concentration before parturition averaged for animals in WT Database.

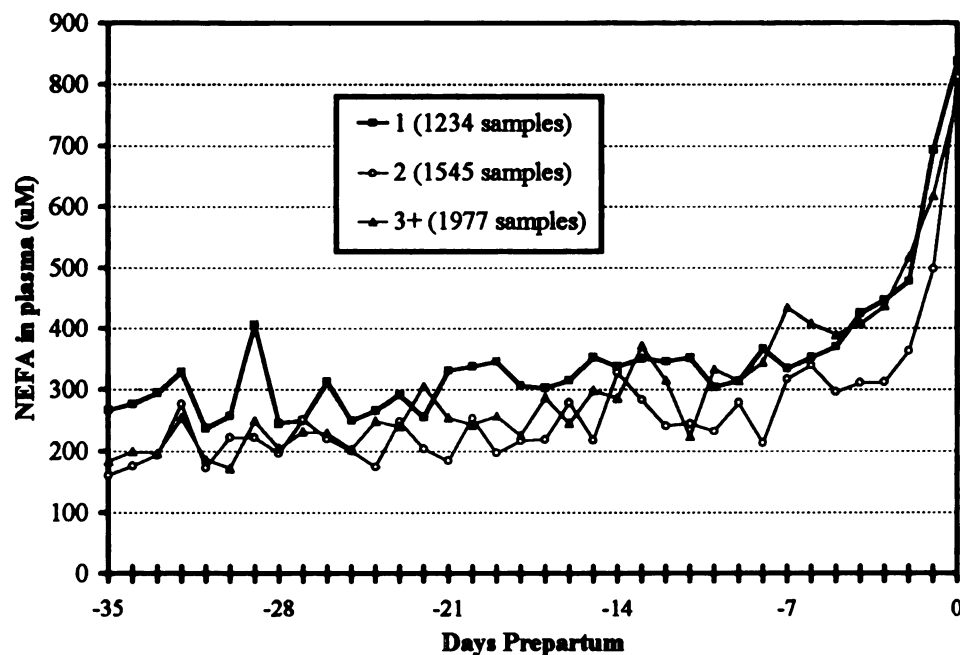


Figure 5. Plasma NEFA concentration before parturition by parity in WT Database.

Relationship of BCS, PTA_m, Parity, Herd and Day prepartum to plasma NEFA

The effects of parity, herd, BCS Group, and day prepartum on the concentration of NEFA in plasma were examined with the GLM procedure in SAS. NEFA was the dependent variable and BCS Group, parity, and herd were blocks and number of days prepartum was used as a covariate to account for the increase in plasma NEFA as animals approached calving. Day prepartum was significant as a covariate only at week -1 (Table 1). Herd, parity, and BCS Group were significant at week -2 and week -1. It should be noted that the variation in NEFA associated with the term “herd” accounts for variation not only in on-farm management and diets, but also for interassay variation among NEFA assays, time of the year, and time the sample was taken relative to feeding. Primiparous

animals had higher NEFA than the other parity groups while parity 2 animals had the lowest plasma NEFA in both week -2 and week -1 (Table 2). The lowest and highest BCS Groups had the highest NEFA in plasma in both week -2 and week -1 (Table 2).

In another analysis, the GLM procedure was used with the NT&PTA_m Database. Again, NEFA was the dependent variable. Parity, BCS Group, PTA_m were used as blocks and day prepartum was used as a covariate. For week -2, PTA_m was not significant in the model (Table 3). At week -1, the means for PTA_m showed the same trend as week -2 (Table 2) and approached statistical significance at $P=.09$ (Table 3). In this model, BCS group was not significant at week -2 or week -1; possibly because there were fewer animals in the dataset.

Table 1. Factors affecting NEFA in plasma at week -2 and week -1 (NT Database)

Source	Degrees of Freedom	Type III SS	Mean Square	Probability
<i>Week -2</i>				
Days prepartum	1	1551	1551	0.8398
Lactation	2	602366	301183	0.0004
Herd	94	18650324	198408	0.0001
BCS Group	3	320492	106831	0.0382
Lactation*BCS Group	6	284899	47483	0.2776
Error	957	36318777	37951	
Total	1063	56178409		
<i>Week -1</i>				
Days prepartum	1	4179062	4179062	0.0001
Lactation	2	1773207	886604	0.0001
Herd	94	26457305	281461	0.0001
BCS Group	3	587288	195763	0.0650
Lactation*BCS Group	6	467257	77876	0.4503
Error	1035	83845951	81011	
Total	1141	117310070		

* Probability of a greater F value occurring by chance

Table 2. LS Means for NEFA concentration by parity BCS, and PTA_m

	Week -2		Week -1	
	n	NEFA (LSMeans)	n	NEFA (LSMeans)
<i>Effect of Parity</i>				
Parity 1	304	338	360	442
Parity 2	351	244	335	319
Parity 3+	409	297	447	425
<i>Effect of BCS</i>				
Very Low BCS	102	312	107	440
Low BCS	246	274	259	353
Medium BCS	448	270	493	374
High BCS	268	317	283	415
<i>Effect of PTA</i>				
Low PTA	183	311	188	426
Medium PTA	475	287	511	371
High PTA	100	272	106	352

Table 3. Factors affecting NEFA at week -2 and week -1 (NT&PTA_m Database)

Source	Degrees of Freedom	Type III SS	Mean Square	Probability
<i>Week -2</i>				
Days prepartum	1	829	829	0.8841
Parity	2	663066	331533	0.0002
Herd	82	13871243	169162	0.0002
BCS Group	3	151591	50530	0.275
PTA Group	2	80170	40085	0.3585
Error	667	26020052	39011	
Total	757	40786951		
<i>Week -1</i>				
Days prepartum	1	1867329	1867329	0.0001
Parity	2	1901948	950974	0.0001
Herd	84	18146779	216033	0.0001
BCS Group	3	190283	63428	0.4925
PTA Group	2	37392	18696	0.0931
Error	712	56254902	79010	
Total	804	78398633		

* Probability of a greater F value occurring by chance

Relationship of PTA_m, Parity, Herd and Day prepartum to BCS

To look at the effect of PTA_m, parity, and herd on BCS, the GLM procedure in SAS was used with BCS as the dependent variable. For one analysis the NT&PTA Database was used with PTA_m Group, parity, and herd as blocks (Table 4), all of which were significant in the model ($P < .01$). Animals in the Low PTA_m Group had significantly higher BCS than animals in the other PTA_m Groups. In a second analysis, the NT Database was used with BCS as the dependent variable, and parity and herd as blocks; this analysis was done to take advantage of the higher number of animals in the NT database. In this analysis, herd and parity were significant ($P < .01$), and primiparous animals had the highest BCS at 3.60, then parity 3+ animals at 3.37, and finally parity 2 animals at 3.23.

Table 4. Effect of PTA_m Group and parity on BCS for the 2 weeks prepartum. PTA_m and parity had a significant effect ($P < .01$) on BCS.

	n	Mean BCS (LSMeans)	SEM (LSMeans)
<i>Effect of PTA Group</i>			
Low PTA Group	264	3.50	.037
Medium PTA Group	755	3.37	.023
High PTA Group	152	3.31	.045
<i>Effect of Parity</i>			
Parity 1	492	3.60	.024
Parity 2	525	3.23	.023
Parity 3+	638	3.37	.021

Time frame of diseases

At the beginning of the study, many farmers were not recording the date on which a health problem occurred. This led to a revision of the health sheet (Appendix C). Farmers recorded the date of a disease incident 62% of the time for retained placenta, 67% of the time for milk fever, 79% of the time for ketosis, and 83% of the time for displaced abomasum. These diseases were reported to have occurred within 60 days after parturition. Of the 192 cases of mastitis, 160 had a date reported. Of these 160 cases with reported dates, 136 occurred within the first 60 days and 102 occurred within the first 10 days. Using animals from the WT Database, ketosis, displaced abomasum, and mastitis occurred an average of 7 ± 0.5 , 12 ± 0.7 , and 47 ± 4 days postpartum respectively (see Figure 6).

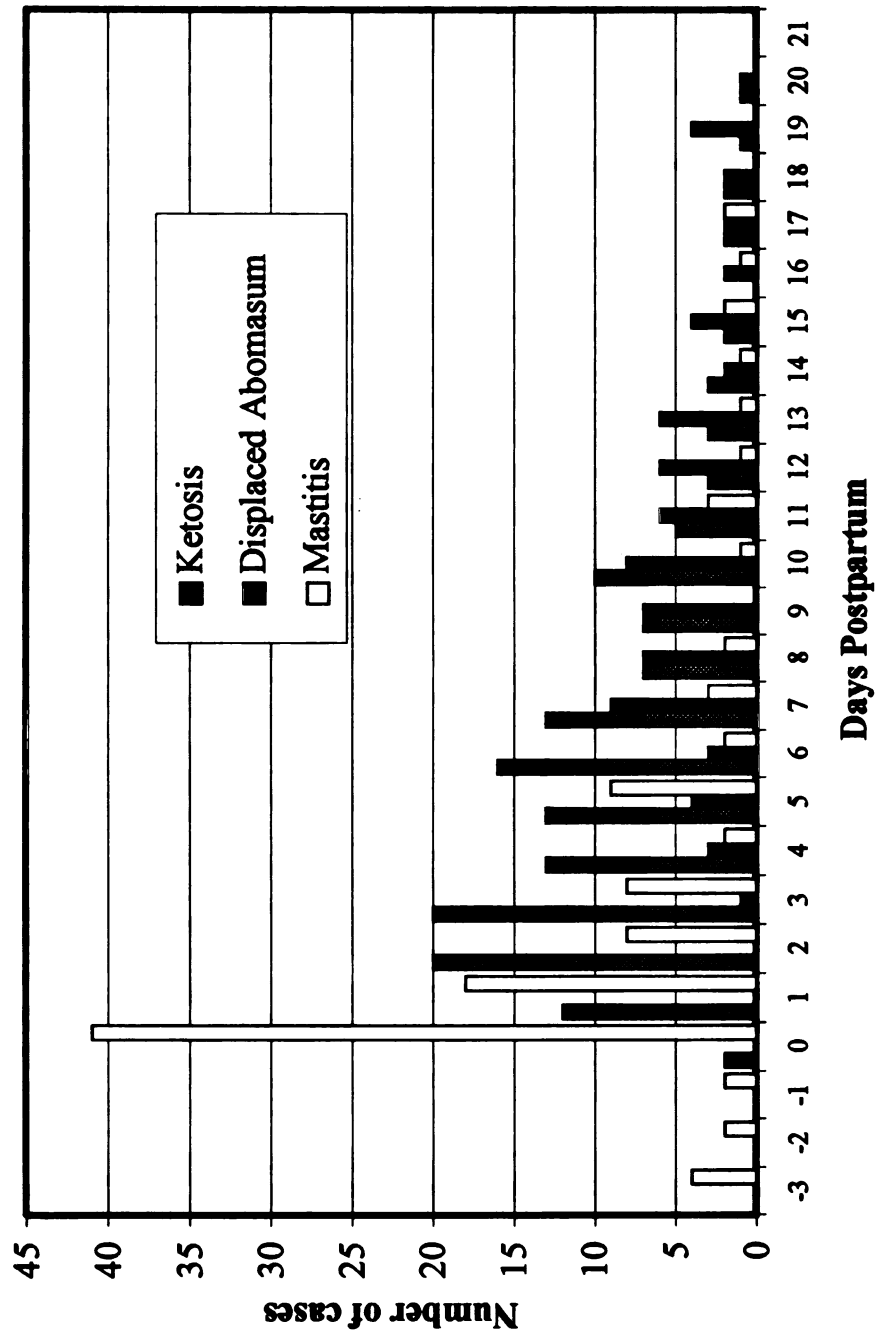


Figure 6. Number of cases of mastitis, ketosis, and displaced abomasum in the first 21 days after calving.

Association of Disease and Parity

Cochran-Mantel-Haenszel (CMH) statistics in SAS and the NT Database were used to examine the relationship of parity and disease. Primiparous animals had a significantly higher incidence of dystocia while multiparous animals had a significantly higher incidence of retained placenta, milk fever, ketosis, and general mastitis (Figure 7). Parity and disease were not significantly associated for displaced abomasum and peripartum mastitis.

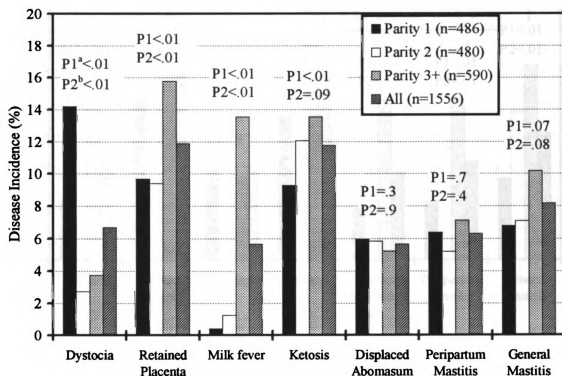


Figure 7. Incidence of disease by parity.

^a P value for the association between disease and parity, controlling for NEFA Group and BCS Group.

^b P value for the association between disease and parity, controlling for no other factors.

Association of Disease and NEFA Group among Parities

The incidence of disease and association with NEFA Group was investigated using CMH statistics and the NT Database. Across parities, the incidence of dystocia, retained placenta, ketosis, displaced abomasum, and mastitis was higher in animals in the High NEFA Group (Figure 8). The incidence of milk fever across all parities was not different among NEFA Groups.

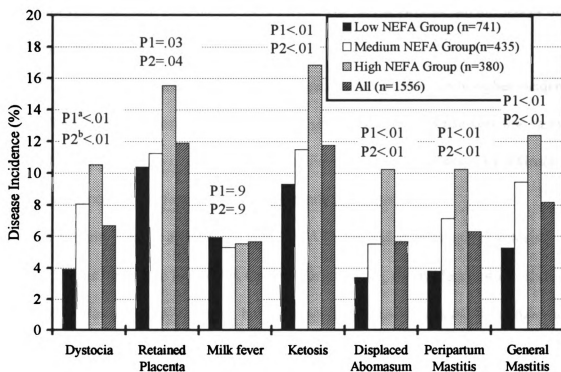


Figure 8. Disease incidence within NEFA Group across parities

^a P value for the association between disease and NEFA Group, controlling for Parity and BCS Group.

^b P value for the association between disease and NEFA Group, controlling for no other factors.

Association of Disease and NEFA Group within Parity

Primiparous animals in the high NEFA Group had a significantly higher incidence of dystocia than animals in the low NEFA Group. However, for parity 2 and 3+, dystocia was not significantly different among NEFA Groups (Table 5).

When sorted by parity, retained placenta and NEFA Group were associated significantly only for parity 3+; animals in the high NEFA group had an incidence of 22.5% for retained placenta while the medium and low NEFA Groups had incidences of 13.0% and 13.7%, respectively.

The high NEFA Group for primiparous animals had a significantly higher incidence of milk fever than the other NEFA Groups. But primiparous animals had very few cases of milk fever compared to multiparous animals. For multiparous animals, NEFA Group and the incidence of milk fever was not significantly associated.

For parity 1 and 2 animals, the high NEFA Group had an incidence of ketosis about 2.5 times that of the low NEFA Group. For parity 3+, the incidence of ketosis also increased as plasma NEFA increased, but this association was not statistically significant. The high incidence of displaced abomasum in the high NEFA Group was 4 times that of the low NEFA Group for parity 2 and 3+. However for primiparous animals, the incidence of displaced abomasum was not different among NEFA Groups. The incidence of mastitis was significantly higher in the high and medium NEFA Groups in all parities than in the low NEFA Group for all parities.

Table 5. The association of disease incidence and NEFA Group by parity.

	Dystocia	Retained Placenta	Milk fever	Ketosis	Displaced Abomasum	Peripartum Mastitis	General Mastitis
<i>-----Parity 1-----</i>							
	Incidence of Disease (% of NEFA Group)						
Low NEFA	8.1	8.7	0.0	5.8	4.1	3.5	3.5
Medium NEFA	15.2	10.1	0.0	8.9	6.3	6.6	7.6
High NEFA	20.0	10.3	1.3	13.6	7.7	9.0	9.7
	<i>Significance of General Association</i>						
<i>P1^a</i>	<i>P=.01</i>	<i>P=.9</i>	<i>P=.06</i>	<i>P=.04</i>	<i>P=.5</i>	<i>P=.09</i>	<i>P=.06</i>
<i>P2^b</i>	<i>P=.01</i>	<i>P=.9</i>	<i>P=.12</i>	<i>P=.05</i>	<i>P=.4</i>	<i>P=.11</i>	<i>P=.07</i>
<i>-----Parity 2-----</i>							
	Incidence of Disease (% of NEFA Group)						
Low NEFA	2.4	8.3	1.4	8.9	3.1	3.1	4.8
Medium NEFA	3.5	10.4	0.9	13.0	7.8	8.7	10.4
High NEFA	2.7	12.2	1.4	23.0	13.5	8.1	10.8
	<i>Significance of General Association</i>						
<i>P1</i>	<i>P=.8</i>	<i>P=.5</i>	<i>P=.9</i>	<i>P=.01</i>	<i>P<.01</i>	<i>P=.04</i>	<i>P=.05</i>
<i>P2</i>	<i>P=.8</i>	<i>P=.5</i>	<i>P=.9</i>	<i>P<.01</i>	<i>P<.01</i>	<i>P=.04</i>	<i>P=.06</i>
<i>-----Parity 3+-----</i>							
	Incidence of Disease (% of NEFA Group)						
Low NEFA	2.9	13.7	14.4	11.9	3.3	4.7	6.9
Medium NEFA	4.3	13.0	13.6	13.0	3.1	6.2	10.5
High NEFA	4.6	22.5	11.9	17.2	11.3	12.6	15.9
	<i>Significance of General Association</i>						
<i>P1</i>	<i>P=.7</i>	<i>P=.02</i>	<i>P=.7</i>	<i>P=.3</i>	<i>P<.01</i>	<i>P=.01</i>	<i>P=.01</i>
<i>P2</i>	<i>P=.6</i>	<i>P=.03</i>	<i>P=.8</i>	<i>P=.3</i>	<i>P<.01</i>	<i>P=.01</i>	<i>P=.01</i>

^a P values for the general association between disease incidence and NEFA Group, controlling for BCS Group

^b P values for the general association between disease incidence and NEFA Group, controlling for no other factors.

Association of BCS Group and Disease Incidence

The association of BCS and Disease was studied using CMH statistics and the NT Database. Across parities, the incidence of ketosis, and displaced abomasum was significantly associated with BCS Group (Figure 9). As BCS increased, the incidence of ketosis and displaced abomasum increased. There was no relationship between BCS Group and the incidence of milk fever, dystocia, retained placenta, and mastitis. The incidence of dystocia was significantly higher in the high BCS group when NEFA Group and parity were not controlled; this significance is due to the fact that primiparous animals have significantly higher BCS and a significantly higher incidence of dystocia. (Table 6)

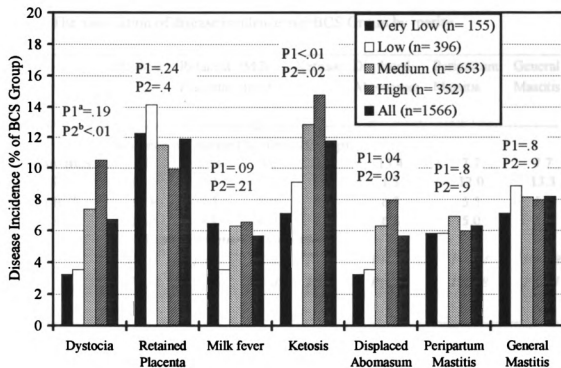


Figure 9. Incidence of disease by BCS Group across parities

* P value for the general association between disease and BCS Group, controlling for Parity and NEFA Group.

^b P value for the general association between disease and BCS Group, controlling for no other factors.

Table 6. The association of disease incidence and BCS Group by parity.

	Dystocia	Retained Placenta	Milk fever	Ketosis	Displaced Abomasum	Peripartum Mastitis	General Mastitis
<i>-----Parity 1-----</i>							
Incidence of Disease (% of BCS Group)							
Very Low BCS	0.0	15.4	0.0	0.0	15.4	7.7	7.7
Low BCS	10.7	9.3	2.7	8.0	1.3	12.0	13.3
Medium BCS	14.8	9.3	0.0	4.9	6.3	5.5	5.5
High BCS	16.2	9.9	0.0	9.3	6.8	5.0	5.6
<i>Significance of General Association</i>							
<i>P1^a</i>	<i>P= .3</i>	<i>P= .9</i>	<i>P< .01</i>	<i>P= .6</i>	<i>P= .20</i>	<i>P= .14</i>	<i>P= .08</i>
<i>P2^b</i>	<i>P= .3</i>	<i>P= .9</i>	<i>P= .01</i>	<i>P= .6</i>	<i>P= .16</i>	<i>P= .18</i>	<i>P= .11</i>
<i>-----Parity 2-----</i>							
Incidence of Disease (% of BCS Group)							
Very Low BCS	2.9	11.4	1.4	10.0	1.4	4.3	7.1
Low BCS	2.4	10.2	0.0	9.6	5.4	4.8	7.2
Medium BCS	2.7	9.1	1.1	13.9	7.0	5.9	7.5
High BCS	3.6	5.4	5.4	16.1	8.9	5.4	5.4
<i>Significance of General Association</i>							
<i>P1</i>	<i>P= .9</i>	<i>P= .6</i>	<i>P= .02</i>	<i>P= .6</i>	<i>P= .4</i>	<i>P= .9</i>	<i>P= .9</i>
<i>P2</i>	<i>P= .9</i>	<i>P= .7</i>	<i>P= .02</i>	<i>P= .4</i>	<i>P= .3</i>	<i>P= .9</i>	<i>P= .9</i>
<i>-----Parity 3+-----</i>							
Incidence of Disease (% of BCS Group)							
Very Low BCS	4.2	12.5	12.5	5.6	2.8	6.9	6.9
Low BCS	1.3	20.8	7.8	9.1	2.6	3.9	8.4
Medium BCS	3.5	15.7	17.0	14.9	5.7	9.2	11.4
High BCS	6.7	11.9	14.8	20.7	8.9	7.4	11.9
<i>Significance of General Association</i>							
<i>P1</i>	<i>P= .14</i>	<i>P= .10</i>	<i>P= .09</i>	<i>P< .01</i>	<i>P< .10</i>	<i>P= .3</i>	<i>P= .6</i>
<i>P2</i>	<i>P= .12</i>	<i>P= .17</i>	<i>P= .21</i>	<i>P= .02</i>	<i>P< .08</i>	<i>P= .3</i>	<i>P= .6</i>

^a P values for the general association between disease incidence and BCS Group, controlling for NEFA Group

^b P values for the general association between disease incidence and BCS Group, controlling for no other factors.

Association of PTA_m and Disease

To study the relationship of PTA_m and disease, CMH statistics were used once again. In a series of analyses, the relationship between PTA_m and disease was studied, controlling for parity, NEFA Group, and/or BCS Group. Ketosis and PTA_m, controlled for NEFA Group and BCS Group, were significantly related at $P=.08$ in primiparous animals. The low PTA_m Group ($n=6$) had an incidence of ketosis of 11%, the medium PTA_m Group ($n=202$) had an incidence of 8.4%, and the high PTA_m Group ($n=62$) had an incidence of 17.7%. Ketosis and PTA_m, controlled for NEFA Group and BCS Group, were not significantly related in multiparous cows. The remaining diseases and PTA_m, controlled for BCS Group and NEFA Group, were not significantly associated within a parity group or across all parities.

When the relationship between PTA_m and disease was tested within and across all parities, controlling only for BCS group, no significant associations were found. When the relationship between PTA_m and the incidence of disease was studied, controlling for no other factors, no significant associations were found within or across parities.

Results from twin analysis

Of the 1655 animals in the WT Database, there were 99 animals that had twins (6.0% incidence). The incidence of twins among parity 1, 2, and 3+ animals was 1.2%, 8.5% and 7.5% respectively. Using the Chi-square statistic, first parity animals had significantly ($P<.01$) fewer cases of twins than second and greater parity animals.

To determine the effect of twins on BCS and NEFA, the GLM procedure of SAS was used. In one analysis, NEFA was the dependent variable, herd and parity were blocks, and day prepartum was used as a covariate. In the second analysis, BCS was the dependent variable, and herd and parity were blocks. Animals with twins had significantly higher plasma NEFA and lower BCS (Table 7).

Table 7. Plasma NEFA and BCS in cows that did and did not have twins

	Week					
	-2			-1		
	n	NEFA	BCS	n	NEFA	BCS
		(LSMeans)			(LSMeans)	
No Twins	1064	283	3.41	1142	382	3.39
Twins	66	534	3.20	79	741	3.11
P<		0.01	0.01		0.01	0.01

Figure 10 shows the incidence of disease in all animals, comparing animals that had twins to animals that did not have twins using a Chi-Square analysis. Animals that had twins had a significantly higher incidence of retained placenta, milk fever, ketosis, and displaced abomasum. However, because multiparous cows have a higher incidence of

twins, and diseases in general, the higher disease incidence may only be related to parity and not the incidence of twins. This is the case for milk fever. Figure 11 shows the incidence of disease in parity 3+ animals separated into twins and no twins. In the parity 3+ group, animals that had twins had a significantly higher incidence of retained placenta, ketosis, and displaced abomasum; however, the incidence of milk fever was not different.

Using CMH statistics and the WT&PTA_m Database, PTA_m was associated significantly with twins, when controlling for parity ($P=.064$). In parity 2, the incidence of twins was 5.6%, 9.3%, and 9.3% in PTA_m Groups 1, 2, and 3, respectively. In parity 3, the incidence of twins was 4.8%, 10.4%, and 10.6% in PTA_m Groups 1, 2, and 3, respectively.

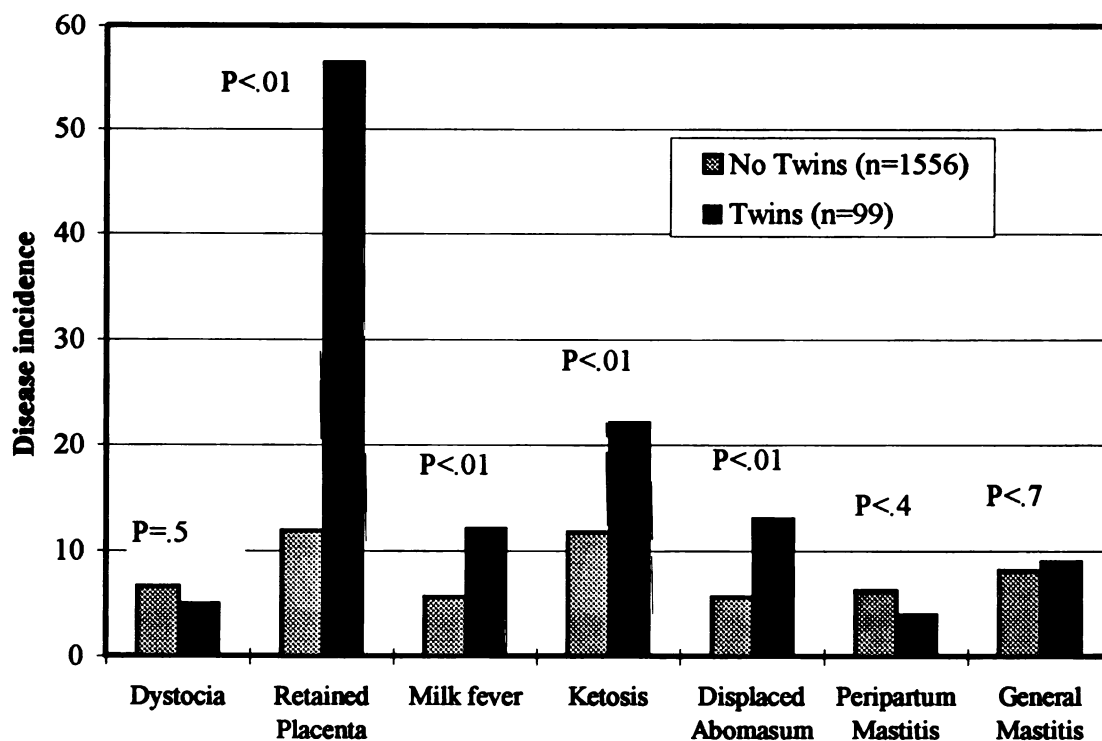


Figure 10. Incidence of disease in animals (n=1655) after giving birth to single or twin calves.

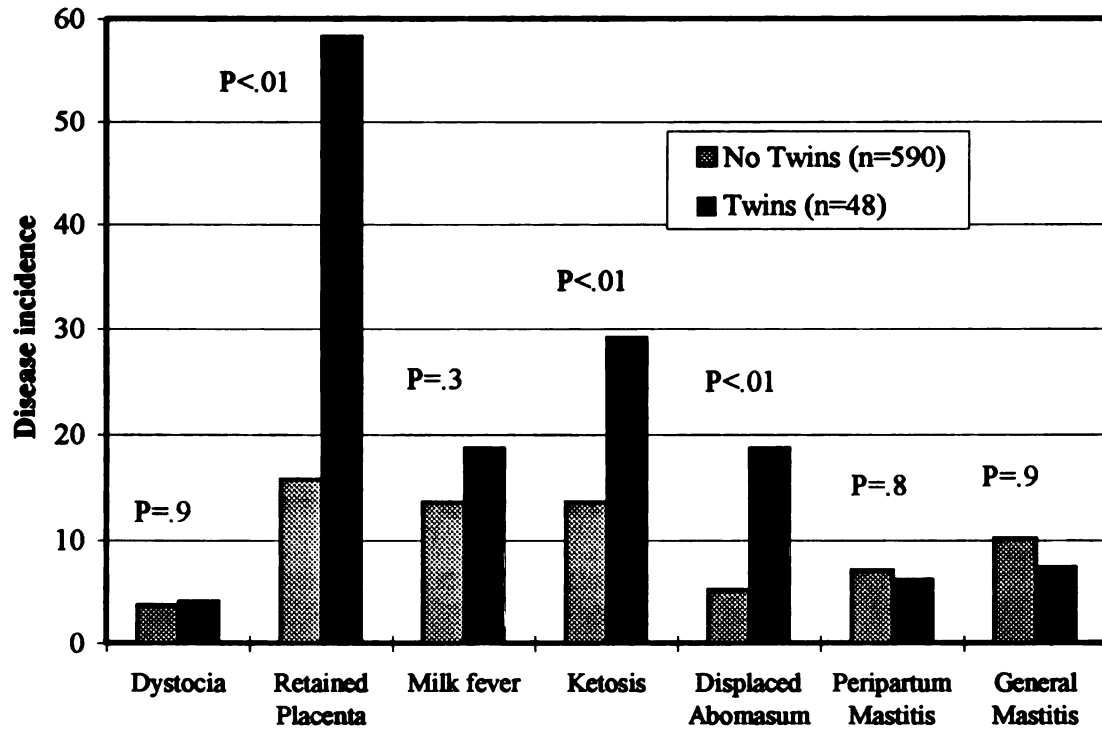


Figure 11. Incidence of disease in parity 3+ (n=638) after giving birth to single or twin calves.

DISCUSSION

Peripartum health problems have caused economic losses on dairy farms for many years. Despite many years of research, the causes of most health problems are still unclear. This study attempted to shed some light on the critical transition period around parturition. The hypothesis of this study was that elevated prepartum NEFA concentration in plasma and fatter body condition are positively associated with more health problems. The results of this study are consistent with this hypothesis. In addition to relationships of NEFA and BCS to health problems, relationships among parity, BCS, and PTA_m were analyzed and integrated into our understanding of the peripartum period (Figure 12).

Effects of Parity on NEFA, BCS and Disease

Plasma NEFA concentration increased over the last few weeks of gestation with a large surge at the time of calving as seen by others (Grummer, 1993; VandeHaar et al.; 1995b; Simmons, 1993). In this study, mean plasma NEFA concentration was different among parities; however, all parities showed a similar surge at calving. Parity 2 animals had the lowest NEFA concentration for the 2 weeks before calving followed by parity 3+, and parity 1 animals.

Interestingly, parity 2 animals which had the lowest NEFA, also had the lowest BCS. Parity 1 animals which had the highest NEFA, also had the highest BCS. How parity influenced BCS is not clear from this study; however, there are several possibilities. Lactating animals may partition less energy toward adipose tissues than non-lactating animals; this would explain why primiparous animals in this study had higher BCS. Perhaps parity 2 animals had lower BCS because they were still growing, as well as producing milk, in the previous lactation. Parity 3+ animals, although they generally produce more milk, are not growing, and because of their larger body size, they may have had higher feed intake and thus partitioned more energy to adipose tissue in the previous lactation.

Not only did NEFA and BCS differ among parities, but the incidence of disease was also different among parities. Primiparous animals had a greater incidence of dystocia as shown by Manfredi et al. (1991). We also found that multiparous animals have a higher incidence of retained placenta, ketosis, and milk fever as others have observed (Emanuelson et al., 1993; Sjollem and Van Der Zande, 1923; Shaw, 1956; Mantysaari et al., 1991). However, multiparous animals did not have a higher incidence of displaced abomasum or mastitis as previously observed (Constable, 1992; Emanuelson, 1993). Perhaps the incidence of displaced abomasum was not different among parities in our study because we did not account for corrective surgeries in previous lactations. Previous corrective surgery probably caused us to underestimate the normal risk of multiparous animals for displaced abomasum.

Relationship of NEFA concentration and Disease

Within and among parities, animals with concentrations of plasma NEFA 25% above average had a higher incidence of health problems. The mechanism for this relationship may be through triglyceride (TG) accumulation in the liver or through immunosuppression associated with poor nutrient balance.

High NEFA levels lead to fat infiltration of the liver (Bell, 1980), so animals with higher NEFA in this study probably had more TG in their livers. Whether this excess fat in the liver caused the higher incidence of ketosis is not clear. Although there have been many studies that associate ketosis and fatty liver, a clear cause and effect relationship has not been established (Grummer, 1993). The most likely explanation for how fatty liver could cause ketosis is that fatty liver impairs liver function. Proper liver function, especially gluconeogenesis, is critical for a cow in early lactation in which glucose flux is 4-5 kg/day (VandeHaar, 1988). Data from Mills et al. (1986), showing decreased gluconeogenic potential in the liver of ketotic animals, supports the idea that fatty liver is the direct cause of ketosis.

In general, when plasma NEFA are taken up by the liver they can be incompletely oxidized to ketones, completely oxidized to CO₂, or reesterified to TG. Since VLDL synthesis is limited in the ruminant, any TG formed in the liver tends to accumulate. Our study shows that there is a moderate rise in NEFA concentration over the last 2 weeks before parturition. This suggests that the development of fatty liver may occur over this 2 week period. This is supported by VandeHaar et al. (1995b) who found that feeding higher energy diets 3 weeks before calving resulted in greater energy intake, lower plasma

NEFA, and less TG in the liver at calving. On the other hand, cows that eat less before calving may simply eat less after calving and thus be more susceptible to ketosis.

Although our data shows that prepartum NEFA concentrations were higher in animals that later developed displaced abomasums, this may not be a direct relationship. One indirect way that the incidence of displaced abomasum could be related to prepartum NEFA concentrations is through the effects of prepartum NEFA on the incidence of ketosis. Displaced abomasum is positively related to the incidence of ketosis (Markusfeld, 1986; Curtis et al., 1985). Descriptors of ketosis include decreased appetite and impaired nerve function (Baird, 1982; Littledike et al., 1981; Sjollem and Van Der Zande, 1923). Either of these could perhaps lead to decreased gut motility which may lead to a higher incidence of displaced abomasum. This is supported by our study where displaced abomasum happened an average of 5 days after the incidence of ketosis and over 50% of the animals that had a displaced abomasum also had a case of ketosis. Of the 40 animals with displaced abomasum, ketosis, and a date for each disease, 36 cases of ketosis happened on the same day or before the day that displaced abomasum was diagnosed.

The high NEFA concentration was also highly associated with retained placenta. One possible mechanism for this is through the effects of nutrient balance on immune system. In this case, the elevated NEFA may have no direct effect on immune function but rather serve as an indicator of poor nutrient balance. Recently, retained placenta has been correlated to a decrease in immune function of the animal (Cai et al., 1994; Gunnink, 1984). If the animals that developed retained placenta were in poor nutrient balance

before calving (as indicated by higher plasma NEFA), their immune systems might have been compromised at the critical time for ejecting fetal membranes after parturition. Immunosuppression has been observed at the time of calving (Detilleux et al., 1994b), but this may be due to altered hormone concentrations, especially increased cortisol (Burton et al., 1995). Whether the suppression is greater in cows that are in poorer nutrient balance before calving is unknown, but it seems likely because malnutrition causes immunosuppression in other species (Keusch, 1981; Rhoads, 1980; Driezen, 1979; Gross and Newberne, 1980). Possible effects of prepartum nutrition on immune function at the time of calving also explain the association between prepartum NEFA and mastitis. Mastitis which is an infectious disease is more likely to occur when the immune system is suppressed (Weigel, 1992), and in fact, the increased incidence of mastitis of animals with elevated NEFA in my study is a good indication that poor prepartum nutrient balance does cause immunosuppression. This connection to the immune system may be why animals with retained placenta are more likely to develop mastitis (Schukken et al., 1989; Emanuelson et al., 1993).

In the case of dystocia, it is difficult to understand the link between increased prepartum plasma NEFA and a higher incidence of problems. The simplest and probably most likely explanation is that an animal in poor nutrient balance has a more difficult time making it through the stressful birthing process.

Relationship of BCS and Disease

Animals with higher BCS had a higher incidence of ketosis and displaced abomasum, even when we controlled for NEFA Group in this analysis. This suggests that

BCS affects disease incidence independent of prepartum lipid mobilization. Perhaps cows with high BCS ate less after calving and thus had more cases of ketosis and displaced abomasum. An association of high BCS and low postpartum DMI has been shown by some researchers (Treacher et al., 1986; Garnsworthy and Topps, 1982) but not by others (Holter et al., 1990).

Causes of elevated prepartum plasma NEFA

There are several factors that affect prepartum NEFA concentrations. One such factor is BCS. We found that, within parity, animals with higher BCS had higher NEFA. Furthermore, BCS and NEFA were both lowest in parity 2 cows and highest in parity 1. The reason that cows with high BCS also had high NEFA is likely that they ate less. High BCS is associated with reduced feed intake postpartum (Treacher et al., 1986; Garnsworthy and Topps, 1982) and prepartum (VandeHaar et al., 1995b). This decline in DMI would increase lipid mobilization resulting in elevated plasma NEFA.

Certainly factors in addition to BCS also affect the plasma NEFA concentration. Some other factors that may alter prepartum NEFA include feeding management, diet quality, housing, temperature, and stress in the last 3 weeks before calving.

Effect of PTA_m on disease, BCS, and NEFA

To ensure that the relationship between NEFA and disease was not an artifact of genetics in our dataset, we looked at the effect of PTA_m on disease, BCS, and NEFA.

On the one hand I expected a positive relationship between PTA_m and incidence of disease. Many studies show a link between high disease incidence and high level of milk

production (Jones et al., 1994; Detilleux et al., 1994a; Simianer et al., 1991). However, the only relationship of disease and PTA_m that was seen in this study was that primiparous animals with high PTA_m developed more ketosis. This relationship is supported by a study with 28,000 Finnish Ayrshire primiparous cows, in which ketosis and milk yield were correlated positively. As in our study, no relationship between ketosis and milk yield existed in multiparous animals (Deluyker, 1991). Perhaps high-producing primiparous animals are more likely to eat insufficient feed for their requirements for both milk production and body growth, and thus develop more ketosis. Despite the fact that the high PTA_m group had significantly lower BCS and lower NEFA than the low PTA_m group, no relationship between PTA_m and the incidence of any disease was observed, perhaps because of the smaller database (NT&PTA Database) with the PTA_m analyses.

Potential problems with this study

One possible weakness of this study is that health problems were diagnosed by individual farmers rather than veterinarians or researchers. Despite the possibility of more incorrect diagnoses, we expected the farmer diagnosis to be a more accurate estimate of the incidence of health problems because farmers do not contact a veterinarian for many health problems. In retrospect, this may have been a problem for ketosis, because farmers did not have a clear description for diagnosis on the health sheets.

Farmer diagnosis of health problems also may have caused problems in understanding the chronology of ketosis and displaced abomasum. Where animals had ketosis and a displaced abomasum, most cases of ketosis were reported to have happened before the occurrence of displaced abomasum. However, this may not be true. For

example, if a displaced abomasum occurred on April 1, the farmer may not have recognized or recorded the displaced abomasum until April 5 (when a veterinarian diagnosed the displaced abomasum). In the period between the actual occurrence of the displaced abomasum and diagnosis/recording of the displaced abomasum, ketosis may have developed. The farmer may have recorded the case of ketosis before April 5, thus indicating that ketosis preceded displaced abomasum, when the opposite may have been true.

Another potential problem with this study is that NEFA may not be the best indicator of energy status and lipid mobilization. One concern is that NEFA concentrations do not remain constant throughout a day. The time relative to feeding affects NEFA concentrations in plasma. Shortly after feeding the concentration of NEFA in plasma are lower than after feeding (VandeHaar et al., 1995a). In our analysis of the effect of BCS and PTA_m on plasma NEFA using the GLM procedure in SAS, herd was used as a block. Using herd as a block probably accounted for some of the differences in NEFA caused by the time of feeding relative to the time of sampling blood. However, in analyses where CMH statistics were used, herd effect was not taken into account and we could not account for time of blood sampling relative to feeding. This increased variation in NEFA that had no relationship with actual daily nutrient balance may have masked some relationships and caused them to be statistically insignificant.

Practical recommendations from this study

Although we did not measure energy intake in this study, energy intake is inversely related to plasma NEFA levels. VandeHaar et al. (1995b) and Sharma et al. (1995)

showed that dry cows that received a diet higher in energy than NRC recommendations tended to have lower plasma NEFA concentrations. This could point to the recommendation that farmers should try to maximize energy intake of the dry cow. This can be done by minimizing the drop in feed intake around calving and/or by increasing the energy density of the feed. Minimizing the drop in DMI is difficult because the causes of the spontaneous decline in DMI are not known. However, maintaining fresh feed in front of the animals for most of the day may help alleviate this problem. Probably a more effective strategy would be for farmers to increase the energy density of dry cow diets in the last 2 or 3 weeks before parturition. The creation of a “close-up” group of dry cows would enable closer supervision of feed and diet management during this critical time. This should result in animals that are in better nutrient balance just before calving, which in turn should decrease the incidence of dystocia, retained placenta, ketosis, displaced abomasum, and peripartum mastitis.

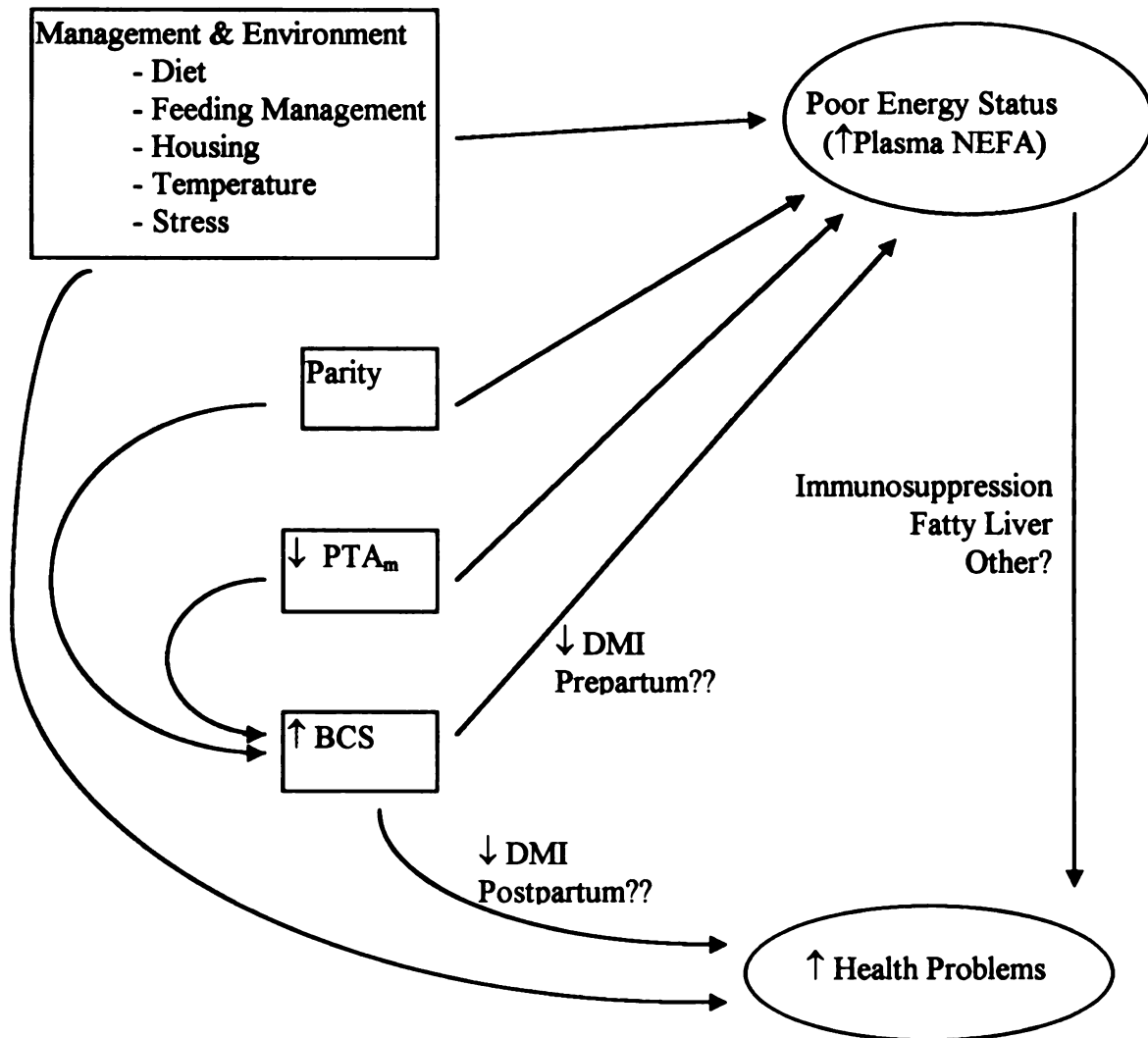


Figure 12. Relationships in the present study and proposed mechanisms for these relationships.

SUMMARY & CONCLUSIONS

In our study higher prepartum NEFA concentrations were associated with a higher incidence of dystocia, retained placenta, ketosis, displaced abomasum, and mastitis but not milk fever. Animals with higher BCS scores had a higher incidence of ketosis and displaced abomasum but not dystocia, retained placenta, milk fever, or mastitis. Prepartum NEFA concentrations were elevated in animals with higher BCS and lower PTA_m and in cows in parity 2 compared to parities 1 and 3+. BCS were lower in animals that had higher PTA_m.

In conclusion, increased lipid mobilization prepartum, as indicated by higher NEFA concentrations in plasma, is associated with more health problems postpartum. Increasing the energy intake of cows prepartum may result in fewer peripartum health problems and lead to more profit for the farmer.

APPENDICES

Appendix A

DECREASING HEALTH AND REPRODUCTIVE PROBLEMS IN DAIRY COWS AFTER CALVING

Will you please take a few minutes to answer the following questions which are designed to insure that we study a range of dry cow management conditions. Individual data will be confidential.

How many cows are you milking? _____

Describe dry cow feeding program: (please circle answer)

Dry cows fed with milking cows	yes	no
One group of dry cows	yes	no
Two groups of dry cows	yes	no
Dry cows fed extra grain close-up	yes	no
Close-up dry cows put with milk cows	yes	no

How many cows have had the following problems in the last 6 mo?

Displaced abomasum _____	Milk fever _____
Ketosis _____	Retained placenta _____
Metritis _____	Breeding problem _____

How do you restrain cows for examination or treatment? _____

Who is/are your veterinarian(s)? _____

Telephone Number : _____

Who is/are your feed dealer and nutrition consultant. Company and Person's name: _____

Do You agree to release your DHIA records to Michigan State University for the duration of this trial?

Yes No

Name (Please Print) _____ Signature _____

Telephone No: _____ Best time to call _____

Fax No. _____

Figure A.1. Initial questionnaire sent to farms

[illegible]

60

Figure B.1. (cont'd)

Formula for mixes (From Computer if available otherwise list as accurately as possible):

Bunk Space per cow:

Dry: _____

Close-up: _____

Calving pen: _____

Fresh: _____

Pen Assignment Criteria:

Dry: _____

Close-up: _____

Calving Pen: _____

Fresh: _____

Describe feed Storage & Amount:

Hay: _____

Haylage: _____

Corn silage: _____

Figure B.1. (cont'd)

Describe Bunk management:

Hours bunk full: _____

Times fed/day: _____

How often Bunks cleaned: _____

Is storage consistent with amounts fed? _____

Describe feed mixing and delivery: _____

DESCRIBE CALVING FACILITIES:

Square feet/cow: _____

Floor type: _____

Bedding: _____

Cows/pen: _____

Avg. days in pen: _____

Criteria for putting in and holding in pen: _____

POSTPARTUM EXAM POLICY (All cows?, if not how selected):**DESCRIBE HEAT DETECTION AND BREEDING:**

Voluntary Waiting period: _____

Who observes: _____

Observed times of day: _____ Heat detection device? _____

AI policy relative to heat: _____

Management of Anestrus Cows (PGF and/or GNRH?): _____

Figure B.1. (cont'd)

COW	FRESH DATE/SEX	HEALTH COMMENTS
1	1/1/1980/F	
2	2/2/1980/F	
3	3/3/1980/F	
4	4/4/1980/F	
5	5/5/1980/F	
6	6/6/1980/F	
7	7/7/1980/F	
8	8/8/1980/F	
9	9/9/1980/F	
10	10/10/1980/F	
11	11/11/1980/F	
12	12/12/1980/F	
13	1/13/1981/F	
14	2/14/1981/F	
15	3/15/1981/F	
16	4/16/1981/F	
17	5/17/1981/F	
18	6/18/1981/F	
19	7/19/1981/F	
20	8/20/1981/F	
21	9/21/1981/F	
22	10/22/1981/F	
23	11/23/1981/F	
24	12/24/1981/F	
25	1/25/1982/F	
26	2/26/1982/F	
27	3/27/1982/F	
28	4/28/1982/F	
29	5/29/1982/F	
30	6/30/1982/F	
31	7/31/1982/F	
32	8/31/1982/F	
33	9/30/1982/F	
34	10/31/1982/F	
35	11/30/1982/F	
36	12/31/1982/F	
37	1/31/1983/F	
38	2/28/1983/F	
39	3/27/1983/F	
40	4/26/1983/F	
41	5/25/1983/F	
42	6/24/1983/F	
43	7/23/1983/F	
44	8/22/1983/F	
45	9/21/1983/F	
46	10/20/1983/F	
47	11/19/1983/F	
48	12/18/1983/F	
49	1/17/1984/F	
50	2/16/1984/F	
51	3/15/1984/F	
52	4/14/1984/F	
53	5/13/1984/F	
54	6/12/1984/F	
55	7/11/1984/F	
56	8/10/1984/F	
57	9/9/1984/F	
58	10/8/1984/F	
59	11/7/1984/F	
60	12/6/1984/F	
61	1/5/1985/F	
62	2/4/1985/F	
63	3/3/1985/F	
64	4/2/1985/F	
65	5/1/1985/F	
66	4/30/1985/F	
67	3/31/1985/F	
68	3/30/1985/F	
69	3/29/1985/F	
70	3/28/1985/F	
71	3/27/1985/F	
72	3/26/1985/F	
73	3/25/1985/F	
74	3/24/1985/F	
75	3/23/1985/F	
76	3/22/1985/F	
77	3/21/1985/F	
78	3/20/1985/F	
79	3/19/1985/F	
80	3/18/1985/F	
81	3/17/1985/F	
82	3/16/1985/F	
83	3/15/1985/F	
84	3/14/1985/F	
85	3/13/1985/F	
86	3/12/1985/F	
87	3/11/1985/F	
88	3/10/1985/F	
89	3/9/1985/F	
90	3/8/1985/F	
91	3/7/1985/F	
92	3/6/1985/F	
93	3/5/1985/F	
94	3/4/1985/F	
95	3/3/1985/F	
96	3/2/1985/F	
97	3/1/1985/F	
98	2/28/1985/F	
99	2/27/1985/F	
100	2/26/1985/F	

This image shows a blank sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

Figure B.1. (cont'd)

Figure B.1. (cont'd)

DRY COW FEEDING FIELD TRIAL CHECK LIST		Date: _____
Name: _____	DHIA: _____	
Recorder(s): _____		

I. Farm Perspective

A. Dry Group

Average time in dry group: _____ (days)

When do they go in? _____

When do they go out? _____

Is there more than 1 dry group (not including CU)? _____

What determines which dry group they are in? _____

Are the heifers in a separate group than the dry cows of similar gestation? _____

Other comments _____

B. Close-Up Group

Does it exist? _____

Average time in close-up group: _____ (days)

When do they go in? _____

When do they go out? _____

Is it part of lactating group? _____

Which one? _____

Are the heifers in a separate group than the dry cows of similar gestation? _____

Other comments _____

C. Calving Pen

Does it exist? _____

Is it part of the Close-Up Group? _____

When do they go in? _____

When do they go out? _____

Other comments _____

D. Fresh Cow Group

Is there a separate group for fresh cows? _____

Is it part of a lactating group? _____

Which one? _____

When do they go in? _____

When do they go out? _____

How are the fresh heifers handled? _____

Other comments _____

Figure B.2. Second version of nutrition and management sheets

E. Milking Group

Describe briefly the groups and when fresh cows enter the groups

Are the heifers handled differently than the cows? How?

Other comments

I. Management Policies**A. Reproduction - for cows on trial (ex. not heifer policy)**

1. Voluntary Waiting Period before breeding _____ (days)

2. Heat Observation

a. how many times per day _____

-average length of observation _____ (min)

b. who observes _____

c. when _____ (times of day)

d. in conjunction with another activity? _____

- what else are they doing? _____

3. Breeding policy

a. AI (farmer or other) or natural _____

b. what sign is breeding time based on _____

(1) standing heat

(2) other _____

c. how many hours after heat are the cows bred _____

d. policy for anestrus cows _____ (1-5)

(1) Vet check and then treatment with _____

(2) prostaglandins without vet check

(3) GNRH without vet check

(4) leave them or sell them

(5) other _____

e. any heat detection devices? _____

- type: _____

B. Feed

1. Storage - Type of Storage and Amount

a. Hay - _____

b. Haylage - _____

c. Corn Silage - _____

d. Other - _____

2. Anionic Salts: Are dry cows fed anionic salts? _____

C. BST

Is it currently being used? _____

When did BST use start? _____

What is use based on? _____

Have any cows on our trial received BST? _____

Which cows? _____

Figure B.2. (cont'd)

III. Group Descriptions

These descriptions are to be done for each group - dry, close-up, maternity pen, fresh cow and milk cow. (Combined groups need only be described once but this should be indicated).

- A. Group Name _____
1. Housing
 - a. Type _____ (1-6)
 - (1) Freestall
 - (2) Covered manure pack with access to outside lot
 - (3) Covered manure pack with no access to outside lot
 - (4) Dry Lot - no cover
 - (5) Pen - size of pen _____ (ft²)
 - (6) Other _____
 - b. Roominess _____ (# of cows/# stalls)
or _____ (# of cows/pen)
 - c. Quality
 - (1) type of bedding _____ (a-e)
 - (a) straw
 - (b) sand
 - (c) paper
 - (d) wood shavings
 - (e) other _____
 - (2) Cleanliness of resting areas _____ (a-c)
 - (a) very clean (mostly bedding)
 - (b) moderate (some manure in bedding)
 - (c) unclean (lot of manure in bedding)
 - (3) Cleanliness of cows _____ (a-c)
 - (a) very clean
 - (b) moderate
 - (c) very dirty
 - (4) General Condition or State of Repair (a-d) _____
 - (a) excellent - well maintained facilities (no broken freestalls)
 - (b) moderate
 - (c) poor - state of disrepair or inappropriate conditions (ex. very small stalls for big heifers)
 - (5) Floor Rating - _____ (a-e)
 - (a) grooved floor
 - (b) very smooth cement floor
 - (c) rough cement
 - (d) straw or manure pack
 - (e) other _____
 - (6) Ease of movement (access to food) _____ (a-c)
 - (a) very good - good cow flow, cows close to feed
 - (b) moderate
 - (c) poor - problems getting to feed, narrow alleys, great distance to feed

Figure B.2. (cont'd)

- (7) Ventilation _____ (a-c)
 - (a) excellent (outdoors/open sidewalls)
 - (b) questionable (closed barn)
 - (c) poor (little air movement)
- (8) Lighting _____ (a-c)
 - (a) very light (outdoors/open sidewalls)
 - (b) moderate lighting
 - (c) poor lighting - dark barn
- 2. Feeding
 - a. Bunkspace _____ (1-3)
 - (1) 1 ft or less/cow
 - (2) 1 to 2 ft/cow
 - (3) >2ft per cow
 - b. Raised Bunk _____ (1 or 2)
 - (1) 1 ft or higher above level of cow's feet
(above cow's hocks)
 - (2) ground level
 - c. Covered bunk - is bunk protected from the
elements such as rain, snow, or sun? _____
 - d. Water availability
 - (1) Plentiful (easy access and numerous troughs)
 - (2) Moderate
 - (3) Poor (limited access and troughs)
 - e. Bunk management _____ (a-e)
 - (1) how often cleaned
 - (a) twice per day
 - (b) once per day
 - (c) once every other day
 - (d) every 3 days
 - (e) other _____
 - (2) how many hours/day do cows have access to
feed bunk _____
 - (3) how many hours/day is there feed in the
bunk _____
 - f. Time of feeding
 - (1) how many times per day cows fed _____
 - (2) how many time per day is feed pushed up
to cows _____
 - (3) what times are cows fed (6am, 6pm etc) _____
 - g. Type of Ration _____ (1-7)
 - (1) Straight True TMR - one feed thoroughly mixed
 - (2) TMR ad lib and limited hay
 - (3) TMR restricted and ad lib hay
 - (4) TMR and grain separate
 - (5) TMR and grain and hay
 - (6) Individual Feeds Fed separately
 - (7) Other _____
 - h. Quality of Ration
 - (1) Does the feed in front of the cows seem
fresh? _____
 - (2) Describe the particle size. _____ (a-c)
 - (a) very course
 - (b) moderate
 - (c) very fine

Figure B.2. (cont'd)

Figure B.2. (cont'd)

Date: _____ Farm: _____

Time Sampled: _____

Group 1 = _____ Time & Day Group 1 last fed: _____

Group 2 = _____ Time & Day Group 2 last fed: _____

Group 3 = _____ Time & Day Group 3 last fed: _____

Group 4 = _____ Time & Day Group 4 last fed: _____

(Cows and heifers within 5 weeks of calving are sampled.)

Group	Cow#	BCS	Health Comments (ex. lameness)
-------	------	-----	--------------------------------

[illegible]

Samples are to be on put ice immediately.

Figure B.2. (cont'd)

Name of Farm:	Location:

[illegible]

Figure C.1. First version of health sheet given to farmers

Appendix D

NEFA PLATE	Photo #:			Date:			Initial:					
	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1119	1371	1470	1546	1284	1447	1544	1287	1498	1541	1610
	82	82	82	82	82	82	82	82	82	82	82	82
	11/09/94	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94
B	1000	1119	1371	1470	1546	1284	1447	1544	1287	1498	1541	1610
	82	82	82	82	82	82	82	82	82	82	82	82
	11/09/94	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94
C	500	1280	1395	1529	1119	1287	1470	1552	1353	1498	1544	Cow C
	82	82	82	82	82	82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	
D	500	1280	1395	1529	1119	1287	1470	1552	1353	1498	1544	Cow C
	82	82	82	82	82	82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	
E	250	1284	1433	1535	1280	1395	1535	1280	1395	1470	1551	Cow C
	82	82	82	82	82	82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94	
F	250	1284	1433	1535	1280	1395	1535	1280	1395	1470	1551	Cow D
	82	82	82	82	82	82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94	11/09/94	11/16/94	11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94	
G	0	1287	1447	1541	NS	1433	1541	1284	1433	1535	1552	Cow D
	82	82	82	82		82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94			11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94	
H	0	1287	1447	1541	NS	1433	1541	1284	1433	1535	1552	Cow D
	82	82	82	82		82	82	82	82	82	82	
	11/09/94	11/09/94	11/09/94			11/16/94	11/16/94	11/23/94	11/23/94	11/23/94	11/23/94	

The First Cow on the plate is: 1119 . The Last Cow on the plate is: 1610 .

Time that colour reagent A was added: _____

Check the side of the plate. Does the volume appear consistent? _____

If not, then which wells are too low or too high? _____

Is there any liquid on top of the lid after shaking? _____

Time that colour reagent B was added: _____

Check the side of the plate. Does the volume appear consistent? _____

If not, then which wells are too low or too high? _____

Is there any liquid on top of the lid after shaking? _____

Time of the final reading: _____

Other comments: _____

Figure D.1. Sheet 1 for NEFA analysis

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1119	1371	1470	1546	1284	1447	1544	1287	1450	1541	1610
B	1000	1119	1371	1470	1546	1284	1447	1544	1287	1450	1541	1610
C	500	1280	1395	1529	1119	1287	1470	1552	1353	1458	1544	Cow C
D	500	1280	1395	1529	1119	1287	1470	1552	1353	1458	1544	Cow C
E	250	1284	1433	1535	1280	1395	1535	1280	1395	1470	1551	Cow C
F	250	1284	1433	1535	1280	1395	1535	1280	1395	1470	1551	Cow D
G	0	1287	1447	1541	NS	1433	1541	1284	1433	1535	1552	Cow D
H	0	1287	1447	1541	NS	1433	1541	1284	1433	1535	1552	Cow D

Plate:

Date:

Initial:

Figure D.2. Sheet 2 for NEFA analysis

Appendix E

```
options ps=63 ls=75 pageno=1;

data one; infile'c:\proj\sapfld\JULY23.prn'missover;
  pi='xxxxxxxx'; bc='xxxxxxxx';
  input hdhia herd cowid $14. cowno @34 calve date9. date date10.
    bcs nefa pi $ @72 visit1 date9. andate date10.
    da rp dyst twins ket mf mas60d died lact ptamilk mas10d;
  daypp=date-calve;
  if lact>2 then lact=3;
  if bcs=0 then delete; if twins=1 then delete;
  if -14<=daypp<0 then output;

data nefa; set one; keep daypp nefa;
proc sort data=nefa; by daypp;
proc means data=nefa noprint; by daypp; var nefa;
  output out=nefa mean=nefa n=n min=min max=max range=range std=std
    skewness=skew kurtosis=kurtosis;
*proc print data=nefa;

data nefa; set nefa; keep daypp nefa; rename nefa=avnefa;
proc sort data=one; by daypp;
data one; merge one nefa; by daypp;
  pernefa=nefa/avnefa*100;

proc sort data=one; by herd cowno lact;
proc means data=one noprint; by herd cowno lact;
  var pernefa bcs da rp dyst twins ket mf mas10d mas60d ptamilk;
  output out=two mean=pernefa bcs da rp dyst twins ket mf
    mas10d mas60d ptamilk;
data two; set two; h=125; l=75;
  if bcs<=2.75 then bc='1Low_BC';
  if 2.75<bcs<=3.25 then bc='2M_BC';
  if 3.25<bcs<4 then bc='3MH_BC';
  if bcs>=4 then bc='4High_BC';
  if ptamilk=9999 or ptamilk<-200 then ptamilk=.;
  if -174<=ptamilk<826 then pta=1;
  if 826<=ptamilk<1826 then pta=2;
```

Figure E.1. Program for CMH Analysis

```

f ptamilk>=1826 then pta=3;
  if ptamilk=9999 or ptamilk<-200 then pta=.;

  if pernefa>h then fagr='3_hfa';
  else if pernefa<l then fagr='1_lfa'; else if l<=pernefa<=h then fagr='2_mga';
  else fagr='xxxx';

proc sort data=two; by fagr;
proc means data=two; by fagr;
  var pernefa bcs da rp dyst twins ket mf mas10d ptamilk;
  title'MEANS FOR NEFA (FAGR) CLASSIFICATION';

proc sort data=two; by bc;
proc means data=two; by bc;
  var pernefa bcs da rp dyst twins ket mf mas10d ptamilk;
  title'MEANS FOR BC CLASSIFICATION';

proc sort data=two; by lact;
proc means data=two; by lact;
  var pernefa bcs da rp dyst twins ket mf mas10d ptamilk;
  title'MEANS FOR LACT CLASSIFICATION';

proc sort data=two; by pta;
proc means data=two; by pta;
  var pernefa bcs da rp dyst twins ket mf mas10d ptamilk;
  title'MEANS FOR PTA CLASSIFICATION';

proc sort data=two; by lact herd cowno;

proc freq; by lact;
  tables bc*fagr*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; by lact; tables fagr*(ket da rp mf dyst mas10d mas60d )/cmh;
proc freq;
  tables lact*bc*fagr*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; tables fagr*(ket da rp mf dyst mas10d mas60d)/cmh;

proc freq; by lact;
  tables fagr*bc*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; by lact; tables bc*(ket da rp mf dyst mas10d mas60d )/cmh;
proc freq;
  tables fagr*lact*bc*(ket da rp mf dyst mas10d mas60d)/cmh noprint;

```

Figure E.1. (cont'd)

```

proc freq; tables bc*(ket da rp mf dyst mas10d mas60d)/cmh;

proc freq;
  tables fagr*bc*lact*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; tables lact*(ket da rp mf dyst mas10d mas60d)/cmh;

proc freq; by lact;
  tables fagr*bc*pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; by lact; tables pta*(ket da rp mf dyst mas10d mas60d)/cmh;
proc freq;
  tables fagr*bc*lact*pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;

proc freq; by lact;
  tables bc*pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; by lact; tables pta*(ket da rp mf dyst mas10d mas60d)/cmh;
proc freq;
  tables bc*lact*pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;

proc freq; by lact;
  tables pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; by lact; tables pta*(ket da rp mf dyst mas10d mas60d)/cmh;
proc freq;
  tables lact*pta*(ket da rp mf dyst mas10d mas60d)/cmh noprint;
proc freq; tables pta*(ket da rp mf dyst mas10d mas60d)/cmh;

proc freq; tables bc*pta*lact*fagr/cmh noprint;
proc freq; tables lact*fagr/cmh;

proc freq; by lact; tables bc*pta*fagr/cmh noprint;
proc freq; by lact; tables pta*fagr/cmh;
proc freq; tables bc*lact*pta*fagr/cmh noprint;
proc freq; tables pta*fagr/cmh;

proc freq; by lact; tables bc*fagr/cmh noprint;
proc freq; by lact; tables bc*fagr/cmh;
proc freq; tables lact*bc*fagr/cmh noprint;
proc freq; tables bc*fagr/cmh;

```

Figure E.1. (cont'd)

```
proc freq; tables fagr*lact*bc/cmh noprint;  
proc freq; tables lact*bc/cmh;  
  
proc freq; by lact; tables fagr*pta*bc/cmh noprint;  
proc freq; by lact; tables pta*bc/cmh;  
proc freq; tables fagr*lact*pta*bc/cmh noprint;  
proc freq; tables pta*bc/cmh;  
  
proc freq; tables bc*fagr*lact*pta/cmh noprint;  
proc freq; tables lact*pta/cmh;  
  
run;
```

Figure E.1. (cont'd)

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