Ketone levels in blood of dairy cows rise naturally after calving. Why does this happen? Milk production is increasing dramatically after calving. The increase in milk production occurs prior to a sufficient increase in dry matter intake, and specifically energy intake. The majority of cows cannot meet their energy requirements for milk production and are forced to mobilize body fat to attempt to meet their energy needs. The fat that is mobilized enters the liver and is converted to ketone bodies when gluconeogenic precursors are limiting.

A rise in ketone levels in blood is not necessarily detrimental to fresh cows. Ketone bodies provide energy to peripheral tissues when carbohydrates are limiting. Therefore, every dairy cow, if she is going to produce enough milk to pay her way, should have rise in blood ketone levels after calving. The situation becomes a problem if dry matter intake does not increase sufficiently in early lactation to support energy needs and reduce the need for fat mobilization.

Subclinical ketosis is defined as increased levels of circulating ketone bodies without the presence of clinical signs of ketosis. Most dairy producers would agree that the costs of subclinical disorders can sometimes be greater than a clinical case. When clinical signs are diagnosed, the cow can be treated and monitored during the following days. Subclinical disorders, when they go unmonitored, can be more costly over the long run because the problem is not caught until it becomes clinical, or it is not caught at all, which will lead to lower milk production for the remainder of the lactation.

Subclinical ketosis has been associated with decreased milk production, impaired reproductive performance, displaced abomasums, metritis, mastitis, and clinical ketosis (Duffield, 2001). Therefore, it is easy to see that economic ramifications of subclinical ketosis can be enormous.

It is important to state that higher producing cows typically have higher levels of blood ketones than lower producing cows (Duffield, 2002). This is not hard to believe. Higher producing cows mobilize more body fat and are at a higher risk of developing subclinical ketosis.

The highest prevalence of subclinical ketosis is in the first two months after calving with the greatest risk being the first two weeks. Several trials and field investigations have been conducted to determine the prevalence of subclinical ketosis in dairy cows. Researchers in Canada determined that herd prevalence for subclinical ketosis is approximately 41% for the first 9 weeks of lactation (Duffield, 2001). The range across 25 herds studied was 8 to 80%. A good
rule of thumb is that the prevalence of subclinical ketosis is approximately 2 to 4 times the incidence rate for cows treated for clinical ketosis (Duffield, 2001).

When lower milk production, increased risk of disease, and reduced reproductive performance are considered, the cost of one case of subclinical ketosis has been estimated to be $78 (Duffield, 2002). If you use a subclinical ketosis prevalence of 41% the economic impact would be about $3200 per year for every 100 cows on your dairy. Therefore, monitoring subclinical ketosis at an early stage in the disease can help minimize economic losses on a dairy.

**MONITORING SUBCLINICAL KETOSIS WITH BLOOD METABOLITES**

It is critical to take enough blood samples to properly draw conclusions. The most common mistake I see is only 1 or 2 blood samples taken, and it is impossible to interpret such a small number of samples. A minimum of 10 to 12 samples should be taken from the eligible group of cows.

**Analyze Nonesterified Fatty Acids in Prefresh Cows**

When cows experience negative energy balance, nonesterified fatty acids (NEFA) increase in blood due to an increase in fat mobilization. The increase in NEFA concentration in blood leads to an increase in liver uptake. Once in the liver, NEFA can be oxidized completely to carbon dioxide, partially oxidized to ketones or reesterified to triglycerides. Cows should stay in a positive energy balance until the last 1 to 2 days prior to calving. During the final 2 days before calving NEFA increase to some degree in all cows, however, the variation between cows is great. Therefore, it is difficult to interpret plasma NEFA levels obtained from samples during the final days before calving.

It is best to use NEFA tests when you already know the herd is having problems with subclinical ketosis. High NEFA’s prefresh identifies ketosis experienced after calving may be due to precalving negative energy balance. According to Dr. Herdt at Michigan State University at least half the prefresh cows between 2 and 4 weeks prior to calving should have NEFA at <325 ueq/L and at 14 to 4 days prior to calving at least half the prefresh cows should have NEFA at <400 ueq/L. According to Dr. Grummer at the University of Wisconsin-Madison, if you sample cows for NEFA on the day of calving at least half the animals should have NEFA at <1000 ueq/L. By three days postpartum animals should have NEFA at <700ueq/L (Herdt, 2003). Cows should be sampled just prior to feeding to capture peak NEFA level.

It is important to remember to use NEFA tests when a higher than desired amount of subclinical and clinical ketosis is occurring. Plasma NEFA is highly variable and high NEFA prefresh does not necessarily mean the animal will have ketosis and fatty liver after calving.

**Analyze Beta-Hydroxy Butyrate in Postfresh Cows**

The gold standard test for monitoring subclinical ketosis is serum beta-hydroxy butyrate (BHB). The three ketones commonly found in blood are acetone, acetoacetate, and BHB. In blood samples BHB is the most stable ketone. Research has determined a cutoff point of 14.4 mg/dl BHB is indicative of subclinical ketosis (Duffield, 2002). Above 14.4 mg/dl BHB, cows are at risk for metabolic disorders. Cows should be sampled for BHB between 2 and 14 days in milk.
Although not well defined, no more than 10% of cows sampled should be above 14.4 mg/dl BHB (Oetzel, 2001). Clinical ketosis is typically defined as 26 mg/dl BHB or more. Cows should be sampled at 4 to 5 hours after feeding to capture peak BHB concentration.

Serum BHB tests are expensive at approximately $5 per sample and it takes several days to ship and analyze the sample, however it is the most accurate and precise tool to monitor subclinical ketosis. Serum BHB is not cost effective or convenient to be used as a cow-side diagnostic test on a daily basis to detect subclinical or clinical ketosis (Carrier, 2003). However, serum BHB may be useful for periodic assessment of the prevalence of subclinical ketosis in a herd.

**MONITORING SUBCLINICAL KETOSIS IN MILK AND URINE**

Cow-side milk ketone powder and urine ketone paper strips marketed in the United States are based on a simple chemical reaction. Acetoacetate reacts with nitroprusside to cause a color change from white to purple. The greater the amount of ketones, the darker the purple color and the higher the degree of ketosis. There is also a milk ketone test available in Canada that has found its way into the United States through Canadian distributors called the KetoTest™ dipstick from Elanco Animal Health that measures BHB levels when dipped in milk. Milk and urine ketone tests are quick and easy cow-side tests, however, quantifying ketone levels is difficult.

Sensitivity and specificity are important measurements to determine the quality of cow-side ketone tests. The sensitivity of a test tells us the proportion of subclinically ketotic cows that test positive. For example, if a test is 80% sensitive, 80% of the cows that were actually subclinically ketotic tested positive and 20% of the cows were wrongly declared nonketotic. The specificity of a test tells us the proportion of nonketotic cows that tested negative. For example, if a test is 90% specific, 90% of the cows that are nonketotic actually tested nonketotic and 10% of the cows were wrongly declared subclinically ketotic.

The University of Minnesota College of Veterinary Medicine recently conducted a trial to evaluate three commonly used cow-side diagnostic tests for the detection of subclinical ketosis in fresh cows (Carrier, 2003). The three test studied were a nitroprusside urine strip (Ketostix® Bayer Corporation), a nitroprusside powder test on milk (KetoCheck™, Great States Animal Health), and a milk BHB test strip (KetoTest™, Elanco Animal Health). The researchers calculated sensitivity and specificity of the tests versus serum BHB.

KetoCheck™ milk test had a poor sensitivity of 42% and high specificity of 99%. When using KetoCheck™ powder expect 58% of the subclinically ketotic cows to go undetected and only 1% of the cows that are nonketotic to be wrongly detected as subclinically ketotic. Ketostix® urine test strips had a sensitivity of 79% and specificity of 96%. KetoTest™ milk BHB test strips had a sensitivity of 75% and specificity of 93%. These results suggest that both the Ketostix® urine strips and KetoTest™ milk BHB strips would detect approximately 3 out of 4 cases of subclinical ketosis and rarely would give a false negative result.

When weighing the results of this study, you have to determine your goals for monitoring subclinical ketosis. If you want to only detect and treat cows that are truly subclinically ketotic and sacrifice treating a few borderline cows that may need treatment tomorrow, the
KetoCheck™ powder milk test would be your choice. Most dairy producers would not choose that option. Most producers want to detect as many subclinically ketotic cows as possible while taking the chance that they will probably detect and treat a few cows that were actually nonketotic. For these producers Ketostix® urine test strips and KetoTest™ milk BHB strips would be the tests of choice.

When deciding between Ketostix® and KetoTest™ strips, producers need to look at availability, cost, and ease of obtaining samples. Ketostix® strips are readily available in the United States while KetoTest™ strips are only available through Canadian distributors and are more expensive. As long as the cow cooperates, obtaining a urine sample from fresh cows is relatively easy. Individual producers will have to decide which strip will work best for them.

**MONITORING MILK FAT:PROTEIN RATIO**

A cow in negative energy balance will mobilize body fat to meet energy needs. A portion of the fatty acids that are mobilized are directly incorporated into milk fat, resulting in an increase in percentage of fat in milk. The percentage of protein in milk will fall slightly in fresh cows because of a reduction in energy supply. Therefore, the ratio of milk fat percentage to milk protein percentage can be a useful tool to monitor the prevalence of subclinical ketosis in your herd. If more than 40% of cows at first DHI test have a fat to protein ratio greater than or equal to 1.5, in a true protein system, the herd may have an elevated level of subclinical ketosis. Fat to protein ratio is fairly good at determining a whole herd problem, but is not sensitive enough for individual cow diagnostics.

**PROTOCOL FOR MONITORING SUBCLINICAL KETOSIS**

Peak incidence of subclinical ketosis occurs during the first two weeks of lactation. Therefore, it is easy to suggest that all cows should be monitored every day during the first two weeks after calving. Blood ketones begin to rise on the day of calving due to a dramatic increase in plasma NEFA at calving. During the first 2 to 3 weeks after calving, ketone levels increase sharply and will become steady or decrease as dry matter intake increases and a more favorable energy balance is achieved (Vazquez-Anon, 1994).

In a perfect world, I would like to have all fresh cows monitored for the first 2 weeks after calving. I would use the urine ketone strips because of their sensitivity, specificity, and ease of sampling. In most cases, dairies will find that 40% of their fresh cows are subclinically ketotic. Each month I would monitor milk fat to protein ratios in my fresh cows. During times when above average numbers of metabolic disorders are occurring I would dig further into the problem. Blood samples should be taken from the prefresh and postfresh cows to analyze NEFA and BHB, respectively, and further define if the increase in metabolic disorders was due to prefresh or postfresh challenges.

In the real world, the proper protocol will vary farm to farm because of variations in facilities. Stall barn cows can easily be monitored during the first 2 weeks after calving. In free stall barns it becomes a question of whether a postfresh group is separate from the rest of the milking herd and, if so, the size of the pen becomes a factor. I like to see postfresh pens sized for cows to be
in the pen for 10 to 14 days after calving. This allows for a sufficient amount of time to monitor fresh cows. Typically, postfresh diets have less energy than high cow diets. It may be detrimental for cows to stay longer than 2 weeks in fresh cow pens. If a separate postfresh pen is not maintained, subclinically ketotic cows may get lost in the shuffle and go undetected until they become clinically ketotic or have a displaced abomasum. Therefore, it becomes critical to determine how you can utilize your facilities as best you can to allow for fresh cow monitoring.

**SUMMARY**

Subclinical ketosis is defined as increased levels of circulating ketone bodies without the presence of clinical signs of ketosis. Ketone levels in blood rise naturally after calving. A rise in ketone levels is not necessarily detrimental to fresh cows. Ketone bodies provide energy to peripheral tissues when carbohydrates are limiting. Therefore, every dairy cow, if she is going to produce enough milk to pay her way, should have a rise in blood ketone levels after calving. The situation becomes a problem if dry matter intake does not increase sufficiently in early lactation to support energy needs and reduce fat mobilization.

Highest prevalence of subclinical ketosis is in the first two months after calving with the greatest risk being the first two weeks. Tools are available to monitor subclinical ketosis to determine cows with the highest risk of becoming clinically ketotic and subsequently having additional metabolic disorders. Ketone levels in urine and milk can be monitored to determine the cows to be treated to increase the chances of a successful lactation.

**REFERENCES**


