Herd-Level Ketosis – Diagnosis and Risk Factors

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Introduction

Since the late 1990's ketosis has emerged as the most important metabolic disease in dairy herds in the US, surpassing ruminal acidosis and milk fever in clinical significance. Fortunately, new testing methods have emerged during the same time period that now allow veterinarians to diagnose ketosis on a herd basis. We have also learned many of the key risk factors for this disease in dairy herds.

It is difficult to subjectively assess the degree of ketosis problems that a herd may be experiencing. Clinical ketosis rates (as determined by dairy producers) are of extremely limited value at all in assessing the true ketosis status of a herd. Herds vary dramatically in their definition of clinical ketosis and in their ability to detect clinical signs in early lactation cows. Producers in smaller herds tend to overestimate the incidence of clinical ketosis (Simensen et al., 1990), and producers in larger herds tend to underestimate the incidence of clinical ketosis (based on my own clinical observations). For these same reasons, attempting to differentiate between cows that have subclinical or clinical ketosis is not very useful. It is essential to make clinical decisions based on the measured prevalence of ketosis in a herd instead of attempting to rely on the dairy producer's perception of clinical signs.

Herds with ketosis problems in early lactation cows also tend to have increased incidence of displaced abomasum (>8%) and increased herd removals in the first 60 days in milk (<8%). Affected herds may also have a higher proportion (>40%) of cows with milk fat to true protein percentages below 0.70 at first test after calving (Duffield and Bagg, 2002). However, none of these clinical findings are definitive evidence for a ketosis problem in a herd. A quantitative evaluation of the prevalence of ketosis is extremely useful in most dairy herds.

The "gold standard" test for ketosis is blood β-hydroxybutyric acid (BHBA). This ketone body is more stable in blood than acetone or acetoacetate (Tyopponen and Kauppinen, 1980). The mostly commonly used cut-point for ketosis is ≥ 14.4 mg/dl (>1400 μmol/L) of blood BHBA. Early lactation cows with blood BHBA concentrations above this cut-point are at threefold greater risk to develop displaced abomasum or clinical ketosis, and cows with blood BHBA concentrations above 19.4 mg/dl (about 2000 μmol/L) are at risk for reduced milk yield (Duffield, 1997). Some studies use a slightly lower cut-point (11.7 mg/dl or 1200 μmol/L) of blood BHBA for defining ketosis. The exact cut-point chosen usually has a minor effect on the interpretation of herd-based results. Clinical ketosis generally involves much higher levels of BHBA – about 29 mg/dl (3000 μmol/L) or more. Some cows have high BHBA without showing any clinical signs, even with careful observation of appetite and attitude.

The BHBA test can be performed on serum samples, and there are no special sample handling requirements. However, blood samples for BHBA should not be collected from the mammary vein. Mammary vein blood is lower in BHBA because the udder tends to extract BHBA but releases acetoacetate (Kronfeld et al., 1968).

Blood BHBA originates from either the liver (due to incomplete oxidation of fatty acids) or from absorption of ruminal butyrate, which is easily converted to BHBA. Blood BHBA concentrations typically increase after feeding (Eicher et al., 1998; Manston et al., 1981) because of BHBA that came from the rumen. Consistent sampling at 4 to 5 hours after the start of feeding has been suggested in order to capture peak BHBA concentrations (Eicher et al., 1998).

It is impractical to determine in a commercial dairy setting, because this would require repeated measures of blood BHBA throughout early lactation. Fortunately, the incidence of ketosis may be inferred from its prevalence. When both ketosis incidence (blood BHBA tests at 1, 2, 3, and 6 weeks after calving) and average early lactation ketosis prevalences were measured in a large field study (Duffield et al., 1998), the cumulative incidence of ketosis (45%) was about 2.2 times the average prevalence of ketosis (20%). In the same study, the reported incidence of clinical ketosis was only 1.5%.

Strategy for Evaluating Herds for Ketosis

Diagnosing ketosis in a herd requires an entirely different diagnostic approach than diagnosing ketosis in an individual cow. Comparison of blood BHBA results from a small number of cows to normal ranges is not appropriate. Herd-based testing is done by subsampling 12 or more animals representative of the animals at risk for ketosis (about 5 to 50 days in milk) and then evaluating the proportion of cows above the cut-point of 14.4 mg/dl. The alarm level for the proportion of cows above this cut-point has not been rigorously defined. Published research studies show an average ketosis prevalence of about 15%, and my own clinical data from herd investigations also shows a ketosis prevalence of 15%. Based on this information, I suggest using 10% as the alarm level for herd-based ketosis testing.

Over the last nine years I have evaluated the ketosis status of 1047 cows in 74 herds as part of my regular clinical service to dairy producers. This is a convenience sampling of herds (not a random sample), and many of these herds had already been identified as potential ketosis problem herds based on clinical signs. The overall prevalence of ketosis in these herds was 15.7%, and 26% of the herds evaluated had ketosis prevalence below 10%. These results indicate that an alarm level of <10% is achievable and appropriate.

Evaluating a minimum sample size of 12 cows per herd is crucial. This sample size requires testing most or all of the eligible cows (i.e., cows between 5 and 50 days in milk) in small to medium-sized herds. A second herd visit is sometimes required before the minimum of 12 cows can be sampled. In larger herds (>250 cows), a suitable sample size is almost always attainable from a single herd visit.

Guidelines for interpreting herd-level blood BHBA results are shown in Figure 1. The mean BHBA concentration for the group is not calculated; instead, the proportion of cows above the cut-point of 14.4 mg/dl is calculated. This proportion is then compared to the 10% alarm level using a 75% confidence interval. Sample sizes of more than 12 cows per herd can give more

confidence that the herd is actually above (or below) the alarm level. However, larger sample sizes obviously entail increased costs. Sampling additional cows is suggested when the results of are close to the alarm level (resulting in a borderline herd classification), or when herd test results are not supported by clinical signs observed in the herd.

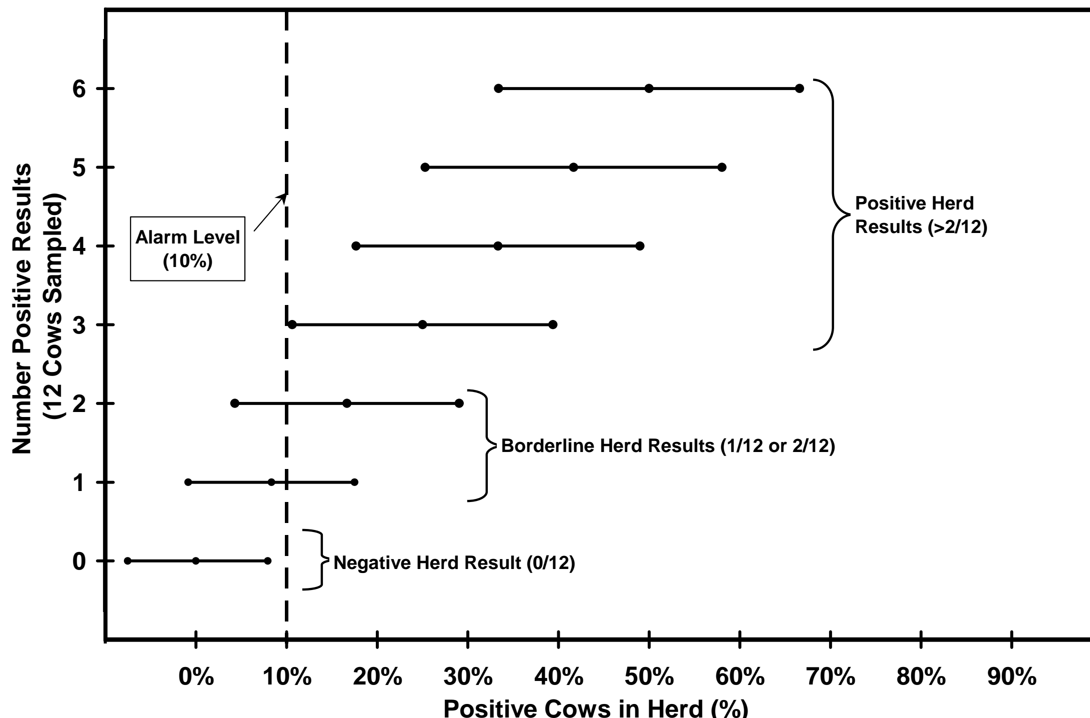

Figure 1. Interpretation of blood BHBA test results using 75% confidence intervals and an alarm level of 10% for test results from 12 cows sampled from within a group 50 cows. Adapted from Oetzel, GR: Monitoring and testing dairy herds for metabolic disease. Vet. Clin. Food Anim. 20:651-674, 2004.

Fifty-three of the 74 herds I have screened for ketosis had sufficient sample size to allow for categorization for ketosis status (see Figure 2). Fourteen herds (26%) were classified as negative for ketosis (i.e., zero cows with ketosis of 12 or more cows tested), 18 herds were classified as borderline, and 21 herds (40%) were classified as positive for ketosis (three or more cows with ketosis of 12 or more cows tested). Each of the 21 positive herds had other clinical evidence to corroborate the ketosis diagnosis.

The ketosis testing strategy described here is designed to identify herds with either very high or very low prevalence of ketosis (Figure 3). It is not intended to 'fine tune' or optimize a transition cow feeding program for ketosis prevention.

About 4% of the cows I have tested for ketosis had very high blood BHBA concentrations (above 30 mg/dl). I only test cows for ketosis that have not been previously identified as sick, so these cows represent missed cases of severe ketosis. While we must allow that a few cows with high blood BHBA may be asymptomatic, most of these cows should be identified as clinically ketotic. Seven herds I have evaluated had more than one cow in the very high BHBA category; these herds were already classified as ketosis problem herds. They also had an apparent problem of inadequate disease recognition in early lactation cows.

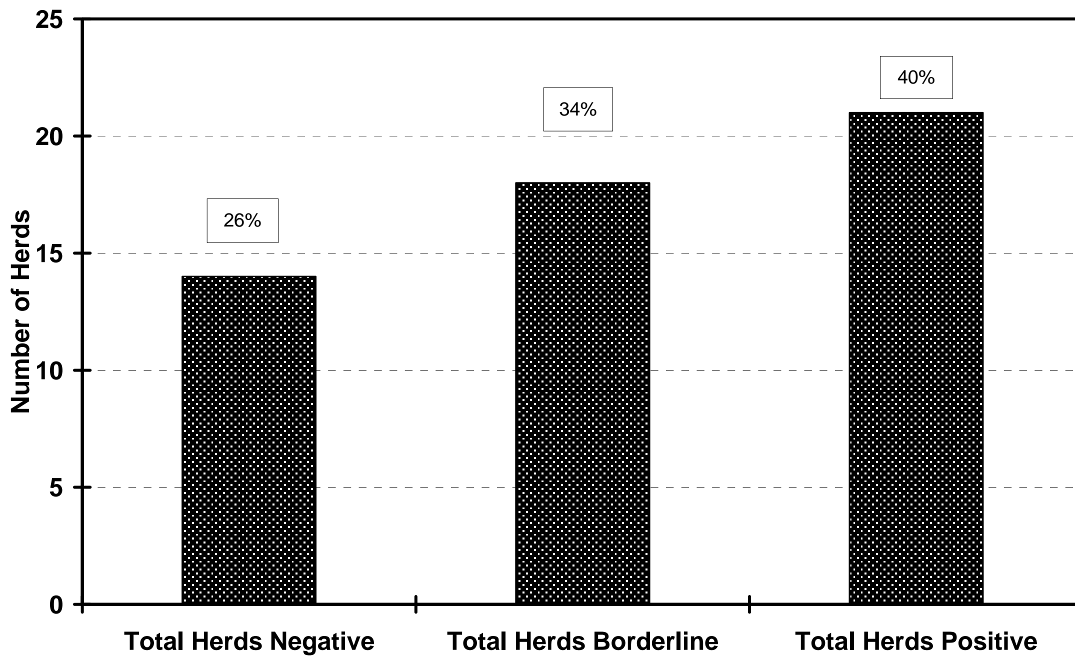

Figure 2. Distribution ketosis status of 53 dairy herds screened by blood BHBA testing for ketosis.

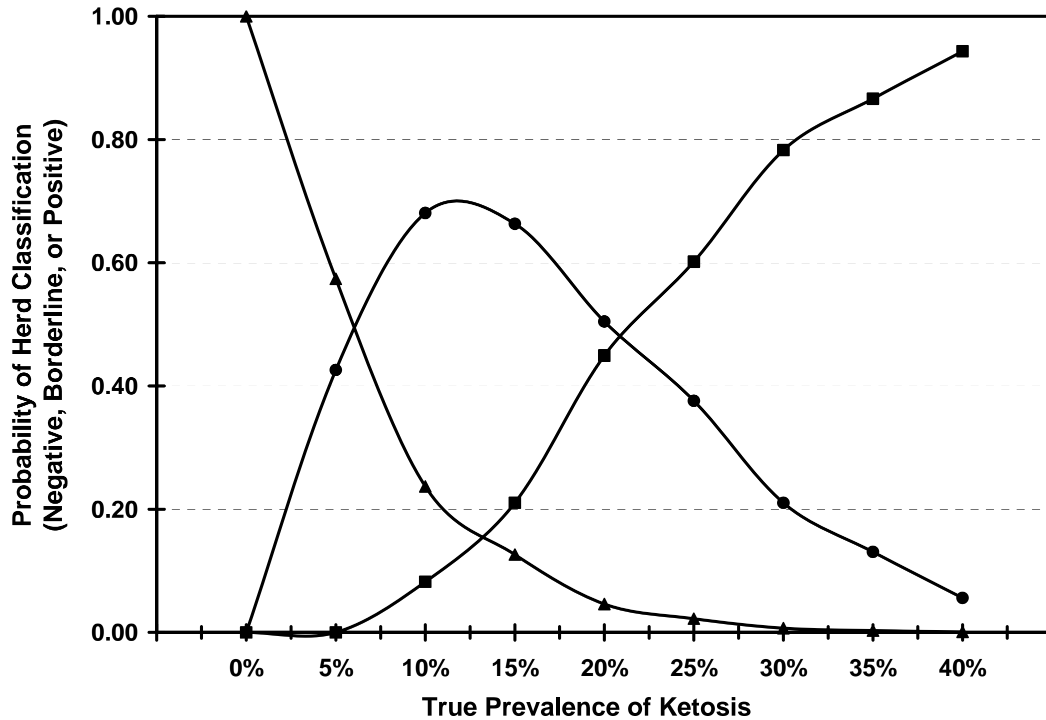

Figure 3. Probabilities of classifying a herd negative (triangle), borderline (circle) or positive (square) for ketosis using a cut-point of blood β-hydroxybutyric acid (BHBA) ≥14.4 mg/dl. The sample size was 12 cows from a group of 50, the alarm level was 10%, and the confidence interval was 75%. For example, a group with a 25% true prevalence of ketosis would have a 2% chance of being classifed as negative, a 38% chance of being classifed borderline, and a 60% chance of being classified as positive for ketosis.

Types of Ketosis in Dairy Herds

I have found it extremely useful in clinical investigations of herd ketosis problems to categorize the ketosis into three general types (Table 1). Each type has a different etiology and therefore a different prevention strategy. There is overlap between the categories, and herds may have a combination of the types. Much of this classification scheme is adapted from Swedish work (Holtenius and Holtenius, 1996) and has been described in detail (Herdt, 2000).

Table 1. Summary of types of ketosis observed in dairy herds

	Ketosis Type:		
Outcome	**Type I**	**Type II**	**Butyric Acid Silage**
Description	Spontaneous; Underfeeding	Fat Cows; Fatty Liver	Wet silages
Blood BHBA	Very high	High	Very high or high
Blood NEFA	High	High	Normal or High
Blood glucose	Low	Low (may be high initially)	Variable
Blood insulin	Low	Low (may be high initially)	Variable
Body condition	Probably thin	Often fat (or may have lost fat)	Variable
Fate of NEFA	Ketone bodies	Liver triglycerides initially, then ketone bodies	Variable
Liver gluconeogenesis	High	Low	Variable
Liver pathology	None	Fatty liver	Variable
Highest risk period	3 to 6 weeks after calving	1 to 2 weeks after calving	Variable
Prognosis	Excellent	Poor	Good
Key diagnostic test	Post-fresh BHBA	Pre-fresh NEFA	Silage VFA analysis
Key intervention	Post-fresh management and nutrition	Pre-fresh management and nutrition	Destroy, dilute or divert the silage

Type I Ketosis. Spontaneous or underfeeding ketosis is the classic form of ketosis that occurs in cows that are 3 to 6 weeks post-calving. It is named type I ketosis because if its similarities to its related metabolic disorder, type I diabetes mellitus. In both conditions blood insulin concentrations are low, although for different reasons. Insulin is low in type I diabetes because of a pancreatic defect, but in type I ketosis insulin is low because of chronic hypoglycemia due to a shortage of glucose precursors.

Type I ketosis occurs between 3 and 6 weeks post-calving because cows are at their highest milk energy outflow at this time. Sometimes these cows simply cannot keep up with energy demand because of some deficiency in nutritional management. They typically did not have difficulties in the pre-fresh period, calved normally, and started their lactation by milking well. Type I ketosis is most common in component-fed herds, because it is very difficult to minimize negative energy balance without causing ruminal acidosis with component feeding.

Cows with type I ketosis are able to make glucose from precursors (mostly propionate from the rumen and amino acids from the small intestine). The limiting factor is the supply of glucose precursors. Blood ketone concentrations become very high and blood glucose concentrations very low under these conditions (Figures 4 and 5).

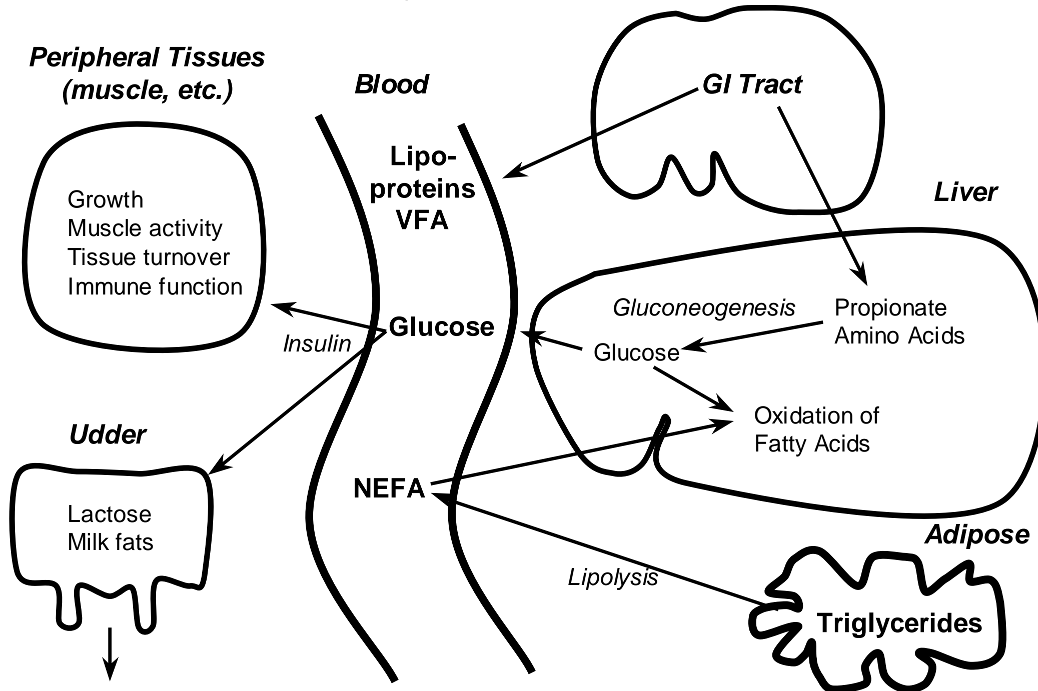

Figure 4. Schematic of glucose metabolism in a normal dairy cow.

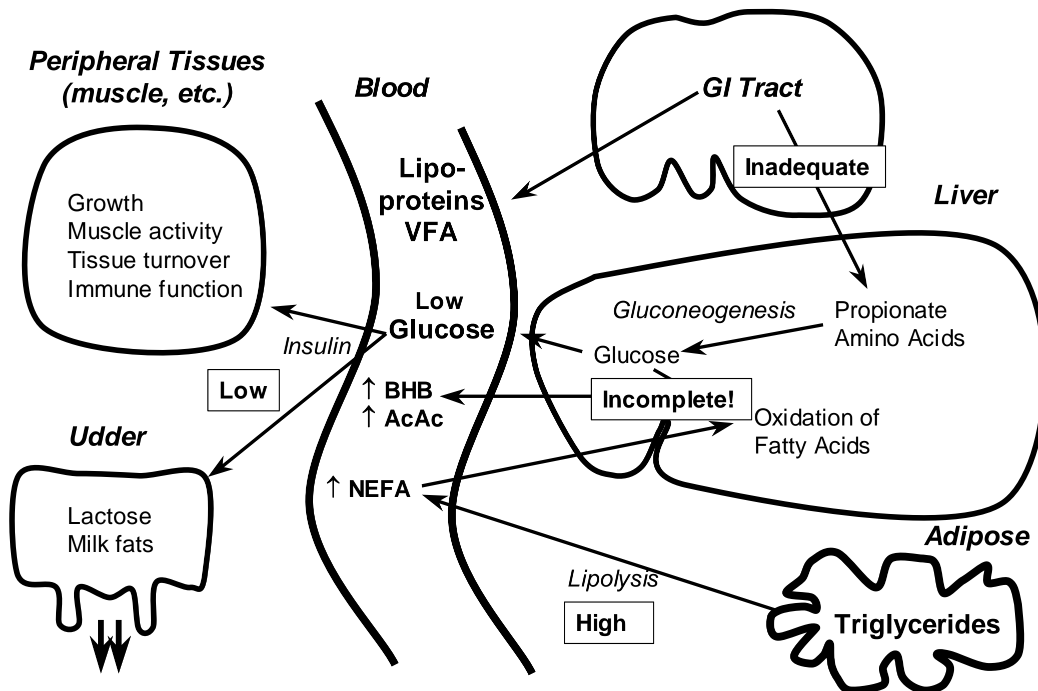

Figure 5. Schematic of glucose metabolism and ketone body formation with type I ketosis.

Cows with type I ketosis generally respond well to a variety of ketosis treatments. All they need is a small boost in their battle to keep up with energy demands, and they are back on track again.

The key to preventing type I ketosis is to maximize energy intake in early lactation. In some herds, this might be as simple as feeding a little more grain in early lactation. Alternatively, a little less grain might be the correct solution if the cows simultaneously have subacute ruminal acidosis (SARA) causing depressed dry matter intake.

Fat supplementation in early lactation does increase energy density of the ration, but is ineffective and contra-indicated for ketosis prevention. Fat supplementation does not provide the glucose precursors needed to fuel gluconeogenesis, but rather floods the liver with more of the fatty acids it is already struggling to oxidize completely. Instead of energy from supplemental fat, post-fresh cows need as much energy as they can reasonably obtain from grains (fermented to propionate and metabolized by the liver to glucose). Fat supplementation also tends to depress dry matter intake, particularly in early lactation. Keep in mind that total energy intake is a combination of both energy density *and* dry matter intake (Table 2). For example, an extra 3 lbs of dry matter intake is worth more energy to an early lactation cow than an increase of .04 Mcal/lb in energy density.

Table 2. Combinations of dry matter intake and energy density to meet energy requirements

	Diet energy density, Mcal NEL/lb							
Feeding Situation	.66	.68	.70	.72	.74	.76	.78	.80
Pre-fresh dry cows, NEL requirement of 18 Mcal NEL/day								
Dry matter needed, lbs/day	27.3	26.5	25.7	25.0	24.3	---	---	---
Early lactation cow, NEL requirement of 40 Mcal NEL/day (after allowing for body weight loss)								
Dry matter needed, lbs/day	---	---	---	55.5	54.1	52.6	51.3	50.0

Over-crowding and/or lack of bunk space can be another cause of insufficient energy intake in early lactation cows. Early lactation cows appear to be especially sensitive to over-crowding; timid or even mildly sick cows in early lactation will have great difficulty getting up to the bunk to eat if they have to fight their way there. Thus, what should be a mild, self-limiting illness for any cause can become clinical ketosis or a displaced abomasum when the post-fresh pen is over-crowded. We recommend a full 30 inches of eating space per cow during early lactation. If 24-inch headlocks are used, then the pen should be stocked to no more than 80% of the number of headlocks – this leaves a net 30 inches of eating space per cow. Other pens on the farm may have restricted bunk space with milder consequences, but restricting bunk space in a post-fresh pen is courting disaster. Six-row barns with three-row pens for post-fresh groups can be particularly difficult to manage unless they are stocked well below stall capacity.

Type I ketosis is uncommon in TMR-fed herds because TMR feeding allows for higher energy intakes with less risk for ruminal acidosis. When type I ketosis does occur in TMR-fed herds, it is usually caused by substantially over-feeding protein and under-feeding energy in post-fresh group. Some post-fresh diets are overly conservative for fiber (because of concerns about SARA) and overly aggressive for protein (in an attempt to get cows to peak higher and

faster). I see problems start when post-fresh NEL is less than about .76 Mcal/lb in combination with crude protein above about 19%. Neither factor by itself causes problems, but the combination does. The main problem with high crude protein content is the energy required to detoxify the extra ammonia that is absorbed from the rumen (i.e., the urea cost). It is not necessary to be overly concerned about SARA in the post-fresh group in TMR-fed herds, because they are being fed a TMR and because their dry matter intakes are still relatively low. The highest risk period for SARA in TMR-fed herds is around peak dry matter intake (90 to 120 days in milk).

Type II Ketosis. This form of ketosis includes the older designation of "fat cow syndrome," but encompasses more than just overly fat dry cows. It includes any cows that develop negative energy balance and begin mobilizing body fat prior to or at calving. Fat cows are at the highest risk for this problem because they are prone to dry matter intake depression around calving (Treacher et al., 1986), but thinner cows are also at risk if nutritional management during the pre-fresh and/or maternity period is poor.

Maintaining positive energy balance up to the time of calving can be difficult, since dry matter intake is naturally depressed for about the five days prior to parturition (Bertics et al., 1992). As for post-fresh cows, maintaining energy intake is a matter of both energy density and dry matter intake. Nutrient densities for pre-fresh groups have to be set with the lowest expected dry matter intakes (i.e., just prior to calving) in mind. Formulating pre-fresh diets for the average intake of the group will result in negative energy balance in those cows approaching calving.

Moving cows to a different pen just prior to calving, over-crowding cows prior to calving, frequent disruptions of pens by adding new cows, moving cows to different pens frequently after calving, and over-crowding after calving are important risk factors for type II ketosis. These issues are discussed in detail in another paper by Dr. Nordlund in these seminar proceedings.

Inadequate maternity pen management also increases the risk for type II ketosis. I often find maternity areas to be over-crowded, dirty, and lacking in eating space. Cows need at least 125 square feet per cow in a maternity pen and generous access to good feed and clean water. Many maternity pens provide no water and/or have very poorly-designed feed bunks (often with a surface lower than the standing surface). It is common to find maternity pen bunks are out of feed for much of the day. If cows spend more than a few hours in a compromised maternity pen, all of the effort spent on preventing type II ketosis in the pre-fresh period is wasted.

The fundamental lesion of type II ketosis is fatty liver (Figure 6). Fatty infiltration of the liver is largely complete by the time of calving, but waits to manifest itself clinically after until calving. It impairs the liver's gluconeogenic capacity, which greatly increases a cow's risk for ketosis once lactation starts. Affected cows develop ketosis within the first week or two after calving. The quality of their post-fresh management has limited bearing on the risk for type II ketosis; affected cows were programmed to get ketosis based on their energy balance and stress just before or soon after calving.

Type II ketosis is named for type II diabetes mellitus, its metabolic counterpart. In both conditions blood insulin concentrations are high and blood glucose concentrations are high (although probably only transiently so in type II ketosis cows). Insulin resistance may also characterize both conditions. Obesity is a particularly important factor in the development of

insulin resistance. Further accumulation of body fat is restricted when tissues are insulin resistant; however, insulin resistance has grave consequences once the cow faces an energy crisis in early lactation and desperately needs to move glucose into her cells. It appears that insulin resistance is not present at the time these cows develop ketosis; however, it may have been a factor in the development of the disease.

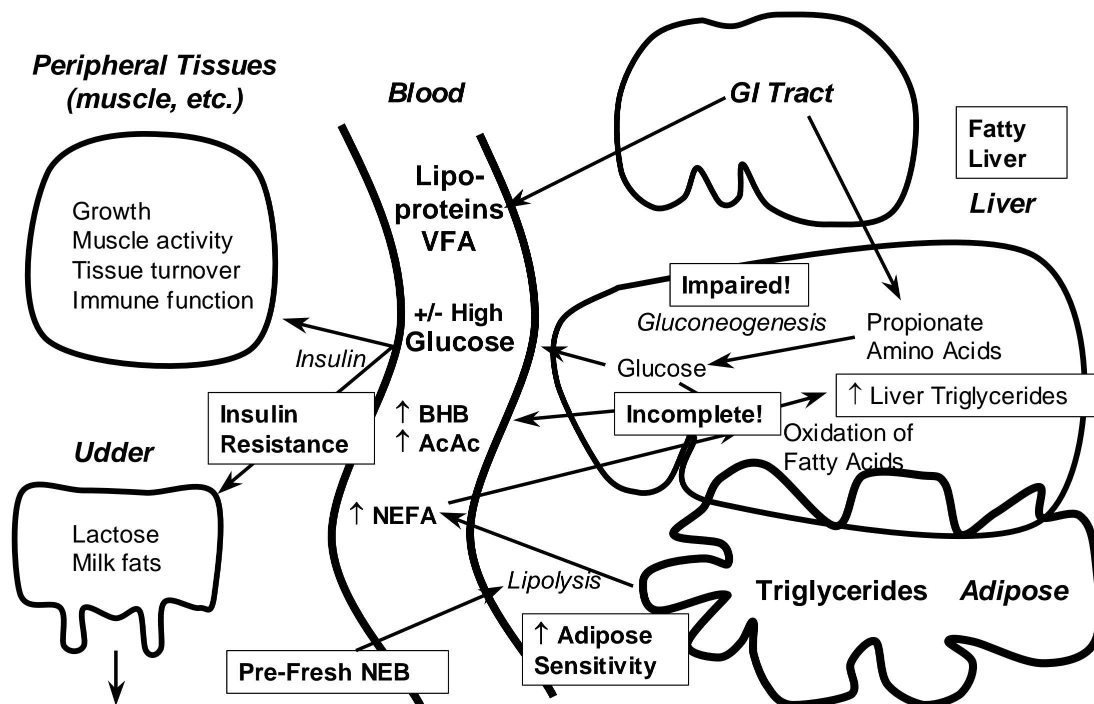

Figure 6. Schematic of glucose metabolism and ketone body formation with type II ketosis.

Obese cows are also prone to increased adipose sensitivity, which is the tendency to mobilize body fat very rapidly under conditions of stress or negative energy balance. This further exacerbates the cow's problems, because excessive mobilization of body fat increases fatty liver infiltration, drives ketone production, and depresses appetite even more. Very fat cows fall into a downward metabolic spiral soon after calving that leads to high mortality.

Obesity in replacement heifers often results in the worst possible cases of type II ketosis in dairy herds. This happens because heifers have more difficulty than cows getting access to feed when they begin to feel sick. Obese heifers are also more prone to dystocia, retained placenta, and metritis than older cows. Mortality in replacement heifers can be high when they are obese and when transition cow management is not excellent.

Blood ketone concentrations are not as high in type II ketosis as for type I. Yet, the prognosis for recovery in type II cases following treatment is poor, because treatment does little to improve the cow's underlying lesion of fatty liver infiltration and loss of gluconeogenic capacity. Cows with type II ketosis often remain ketotic for 1 to 3 weeks.

Besides impairing gluconeogenic potential, fatty liver also impairs immune function by hepatocytes. Severe negative energy balance also suppresses immune function by itself, because immune cells and functions are voracious consumers of energy. The net result is a cow that is

not only persistently ketotic, but also immune suppressed. Many cows with type II ketosis die from infections (metritis, mastitis, pneumonia) that their immune systems would normally have been able to combat.

Type II ketosis is diagnosed in a dairy herd by finding a high incidence of ketosis in cows in the first two weeks of lactation, combined with finding a high prevalence of elevated blood NEFA concentrations in the pre-fresh cows. Other factors help substantiate the diagnosis; these include including obesity, very persistent ketosis, high rates of displaced abomasum, and high mortality rates in early lactation.

Type II ketosis is prevented by excellent pre-fresh nutritional management combined with prevention of obesity in dry cows. Preventing negative energy balance prior to calving requires good dry matter intakes as well as proper energy density of the pre-fresh diet (Table 2). As for the post-fresh cows, nutritional management issues that increase dry matter intake are of more practical importance than increasing diet energy density. For example, increasing dry matter intake by 3 pounds per day increases total caloric intake as much as increasing energy density from .66 to .74 Mcal/lb NEL. Over-crowding pre-fresh pens or restricting bunk space for pre-fresh cows has the same dire consequences as it does for post-fresh cows.

Adding chopped straw (or chopped dry, coarse hay) to pre-fresh diets is popular in some areas of the US. Straw can be an excellent addition to pre-fresh diets, provided that dry matter intake is already very good in the pre-fresh group (>30 lbs/cow/day if lactation 2 or greater cows, and >26 lbs/cow/day if springing heifers). Never add straw to a pre-fresh diet if total caloric intake is already low. Total NEL intake should be between about 17 to 19 total Mcal of NEL for mixed parity pre-fresh groups. Adjust the amount of straw (and concentrate) feeding to achieve this target.

Straw added to pre-fresh diets must be very clean and chopped very finely. Cows typically sort against straw particles over about 1 inch in length. Problems that could result from sorting straw particles in a TMR are vastly more important than the potential for long straw particles to prevent ruminal acidosis. All pre-fresh cows are at low risk for ruminal acidosis because of their relatively low dry matter intakes. Additionally, straw particles are very buoyant in the rumen and probably contribute to mat layer formation even if less than 1 inch in length.

Butyric Acid Silage Ketosis. Some herds have persistent ketosis problems that are caused by feeding ketogenic silages (Tveit et al., 1992). Hay crop silages that are chopped too wet (insufficient wilting time or direct-cut silages) or that are low in water-soluble carbohydrates favor growth of *Clostridium sp.* bacteria. These bacteria ferment some carbohydrates to butyric acid instead the desired lactic acid. Corn silage or ensiled corn grain rarely supports clostridial growth, presumably because of their relatively high water-soluble carbohydrate content. Some grass silages (particularly ryegrass) are also resistant to clostridial fermentation because of their abundance of water-soluble carbohydrates. Time of day at cutting also affects water-soluble carbohydrate content, with the highest concentrations occurring in the hottest part of the day (afternoon). However, it is often impractical to limit forage cutting to just a few hours each day.

Silages that have undergone clostridial fermentation are easy to recognize because of the distinctive odor of butyric acid and protein degradation products that accompany this fermentation pattern. A silage fermentation (VFA) analysis can confirm the presence of and the

amount of butyric acid present in the silage. Appendix I and II contains more information about silage fermentation analysis.

A review of the papers published on silage butyric acid (Appendix III) suggests that daily doses of over 50 to 100 g of butyric acid can cause ketosis, and that doses over about 200 g of butyric acid may induce severe ketosis. About 450 to 950 g of butyric acid will reliably induce severe ketosis in nearly any early lactation cow. High-producing cows in early lactation in modern dairy herds are probably at inherently higher risk for ketosis than the cows used in these older experiments. Thus, be very conservative in recommending how much dietary butyric acid a cow can "handle."

Cows are equipped to metabolize the butyrate produced by ruminal fermentation (about 750 g/day), mostly by using it as metabolic fuel for the ruminal musculature. About 75% of the additional ruminal butyrate is converted to blood BHBA, the direct cause of ketosis (Figure 7). The liver then can convert BHBA to AcAc and vice versa. Thus, there is no "safe" dose of dietary butyric acid for dairy cows. *Any* amount of additional dietary butyric acid increases a cow's risk for ketosis. Whether or not dietary butyric acid causes ketosis depends on the dose of butyric acid consumed and on whether other risk factors for ketosis (early lactation, high production, low dietary energy, high dietary protein, ruminal acidosis, etc.) are also present.

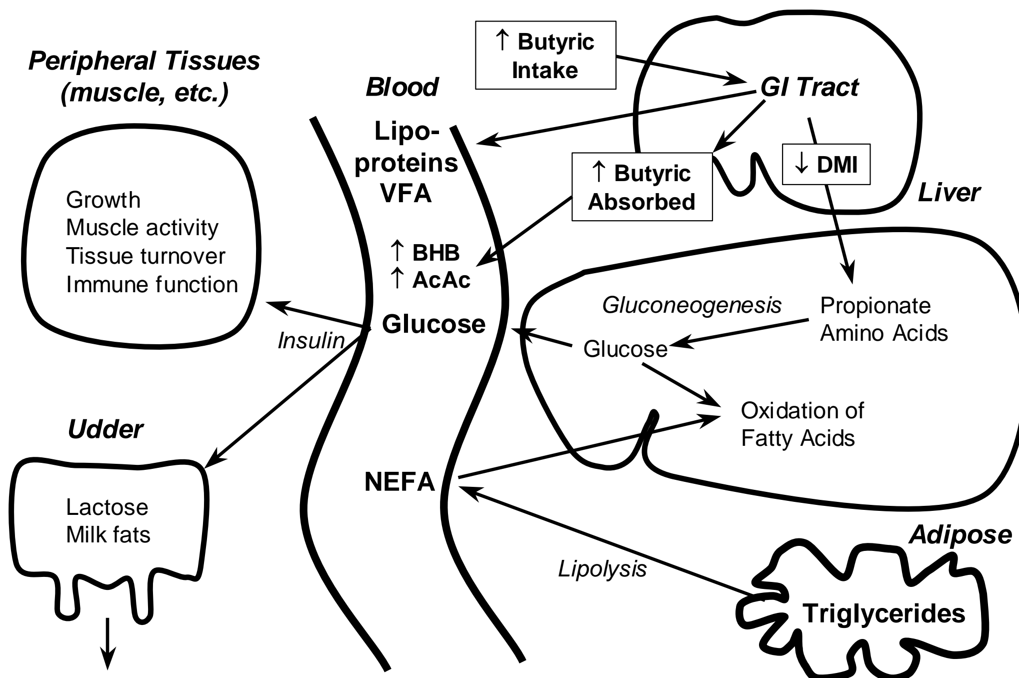

Figure 7. Schematic of glucose metabolism and ketone body formation in a dairy cow consuming excessive butyric acid from silage.

Dairies with large amounts of high butyric acid forage already in inventory have three options - divert, dilute, or destroy it. The first option is to divert this feed away from the pre- and post-fresh cows. It can be fed to replacement heifers, late lactation cows, and/or far-off dry cows. However, the concentration of butyric acid in the forage should be monitored frequently and feeding rates adjusted to keep the daily dose of butyric acid below 50 g/cow/day. Even if

these guidelines are followed, feeding silages containing butyric acid could impair dry matter intake (even if ketosis does not result).

In order to stay below 50 g/day of butyric acid with hay-crop silage as the sole forage (22 lbs forage dry matter/cow/day), the butyric acid content of the hay crop silage cannot exceed .50% on a dry matter basis. If the hay-crop silage makes up half of the forage dry matter (11 lbs/day) and the other half of the forage contains no butyric acid (corn silage or dry hay), then the hay crop silage should not exceed 1.00% butyric acid on a dry matter basis.

"Aerating" the forage prior to feeding it will volatilize some of the butyric acid (perhaps about 50% of the total) and make it safer to feed. However, the butyric acid content of the silage must still be monitored after aeration. Aerated silage should also be watched for heating, although this is unlikely because butyric acid greatly limits aerobic spoilage.

Only under the most the extenuating circumstances should silage containing butyric acid be fed to pre- or post-fresh cows. Even then it must be well-diluted, and the dose of butyric acid fed to the cows should be kept well below 50 g/cow/day. Early lactation cows being fed silages known to contain butyric acid should be intensively monitored for ketosis and promptly treated as needed.

Silages that are over about 2% butyric acid (DM basis) are best destroyed (i.e., hauled away in a manure spreader to be applied to the fields). These silages have value only as fertilizer. It is better to buy new forage than to attempt to feed this silage and deal with the adverse cow health consequences.

Herds with butyric acid silages must modify their future harvesting practices to prevent ensiling overly wet forages in the future. This requires adequate wilting time in the field after cutting and prompt covering of bunker silos if it rains during the filling and packing process. It is useful to recognize that clostridial fermentation of silages does not reach a terminal pH, which means that the fermentation bacteria continue to grow and produce butyric acid indefinitely. In contrast, a normal fermentation by lactic acid bacteria drops the silage pH below the terminal pH, which effectively stops microbial growth and stabilizes the silage mass. If a producer has already harvested a very wet hay crop silage, it should be fed out (or discarded) sooner rather than later. Be especially vigilant for high butyric acid silages in the late winter and early spring months, because hay crop silage that has been in storage the longest time period is often fed at this time of year.

Clinical Data - Types of Ketosis in Dairy Herds

Our clinical experience with BHBA testing in problem dairy herd investigations supports the theory that these three different types of ketosis are present and can be diagnosed in dairy herds. Days in milk at the time of elevated BHBA concentration is especially helpful in making this determination (see Table 3 for examples). Cows with high BHBA concentrations within the first 14 days in milk are most likely to have Type II ketosis. Cows with Type II ketosis may have lingering ketosis past 30 days in milk, but the onset of their ketosis is typically in the first 14 days after calving. Focus your attention on pre-fresh and maternity pen management in herds with type II ketosis. Cows whose BHBA concentrations first rise later than 14 to 21 days after calving probably have Type I ketosis. Focus your attention on post-fresh nutritional management in these herds.

Table 3. Examples of dairy herds with days in milk of cows with high BHBA concentrations (cut-point of ≥14.4 mg/dl) suggestive of either type II or type I ketosis.

Type II Ketosis Example Herd:			**Type I Ketosis Example Herd:**		
Cow	**Days in Milk**	**BHBA (mg/dl)**	**Cow**	**Days in Milk**	**BHBA (mg/dl)**
1902	5	**14.5**	Sheila	5	7.6
2910	5	**25.6**	Susan	5	9.0
4176	8	**43.3**	Lynn	6	4.6
3503	9	6.9	Sparkle	6	7.9
5293	9	**17.8**	Swish	7	9.8
6576	10	**19.9**	Dimples	8	5.5
6624	11	11.3	Gracie	10	4.0
3548	13	11.0	Marcy	10	12.3
3553	13	7.0	Diane	11	8.4
4019	13	4.1	Tootsie	11	8.6
1709	14	**26.1**	Sasha	15	10.0
4081	14	6.7	Olive	19	**23.3**
6662	14	**16.3**	Merry	20	**17.6**
6476	19	13.5	Kristyn	24	**15.7**
6681	23	**18.7**	Morgan	25	**30.6**
4040	26	18.7	April	31	**22.9**
3579	31	8.2	Twinkle	35	4.6
4109	32	6.8	Sarah	38	**23.0**
	Group Summary:	**8/18**		Group Summary:	**6/18**

BHBA = Blood β-hydroxybutyric acid (values over the 14.4 mg/dl cut-point are bolded).
Data points are from clinical herd investigations at the University of Wisconsin-Madison, 2004.

Herds with butyric acid silage ketosis appear to have increased risk for ketosis at any time before 50 about days in milk. This type of ketosis probably interacts with Type II and or Type I problems in the same herd.

Clinical data from our problem herd investigations illustrates a biphasic distribution of ketosis by days in milk (Figure 8). It is possible that there is a time of reduced risk for ketosis between about 15 to 30 days in milk (the time between risk periods for type II and type I ketosis).

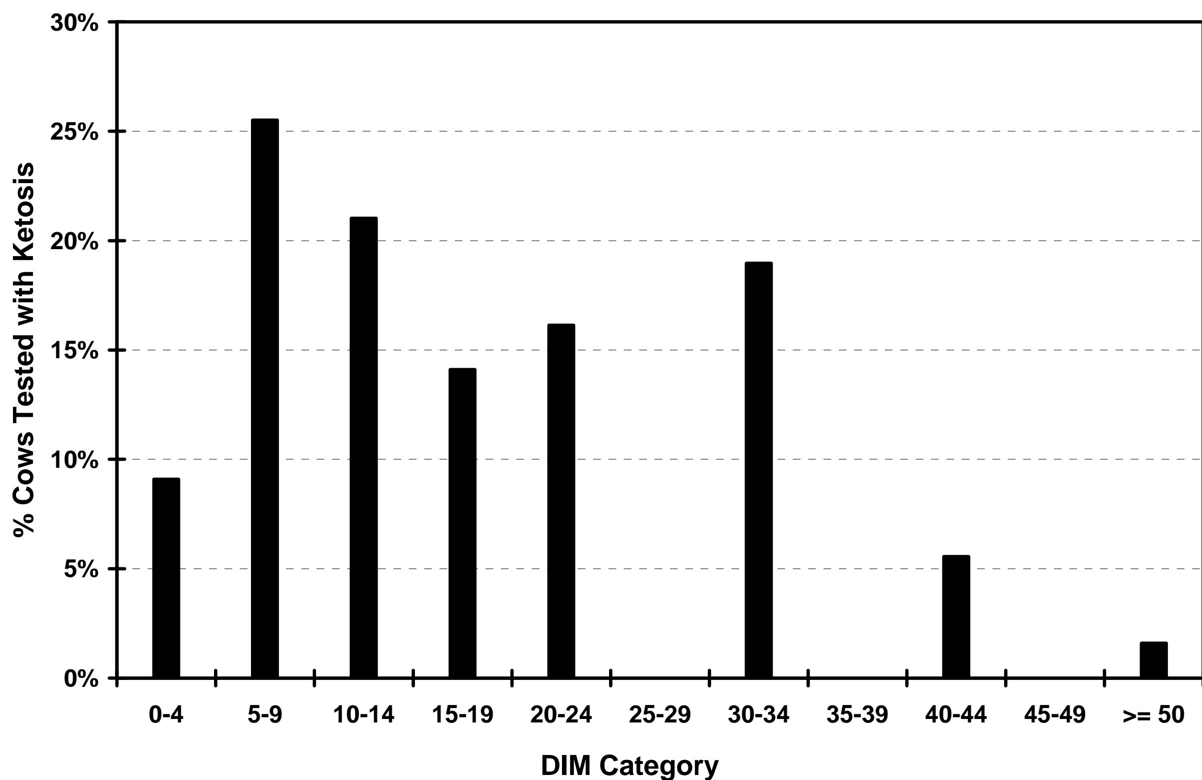

Figure 8. Distribution of ketosis in cows by days in milk. Data are from 1088 cows in 73 herds.

Cowside Tests for Ketosis

A variety of cowside tests are available for ketosis monitoring of dairy herds. However, none of the cowside tests have perfect sensitivity and specificity compared to blood BHBA (see Tables 4 through 6). Therefore, the gold standard ketosis test (blood BHBA) is the most accurate for herd monitoring, and is particularly warranted for investigating herds with presumptive ketosis.

Cowside ketosis tests have the advantages of lower cost, less labor, and immediate results when compared to blood BHBA testing. This makes them particularly useful for making (or excluding) a clinical diagnosis of ketosis in individual, sick cows. However, testing herds for ketosis requires a very different testing strategy compared to diagnostic decision-making for sick cows.

Cowside Urine Tests for Ketosis. Urine can be evaluated for cowside ketosis testing; however it is much more difficult to collect a urine sample than a cowside milk sample. And even with considerable effort, some cows inevitably fail to urinate within a reasonable time period and cannot be tested at all. In research trials, urine samples are not usually collected from 100% of eligible cows. An example is a recent study in which urine samples were successfully collected from only 64% of eligible cows (Osborne et al., 2002). This is a substantial practical limitation on farms and greatly increases labor costs for testing.

Urine acetoacetate can be evaluated quantitatively by nitroprusside tablets (Acetest; Bayer Corp. Diagnostics Division, Elkhart, IN). This test has excellent sensitivity but poor specificity (Nielen et al., 1994) – see Table 4. This makes it a useful test for evaluating individual sick cows (for whom a false positive result is preferred to a false negative one), but not very useful for herd-based monitoring.

A dipstick designed for evaluating milk BHBA has been evaluated for use with urine (Osborne et al., 2002), despite lacking a label for use with urine. As for the urine tablets, this test has good sensitivity but poor specificity (Table 4). The higher cost of these strips compared to other urine ketone tests makes them impractical for use on urine, although they are an excellent cowside test for milk BHBA, as described later.

Table 4. Sensitivity and specificity of urine cowside tests compared to blood BHBA (cut-point of ≥14.4 mg/dl).

Test type / Study	Herds Tested	% Ketosis	Total Samples	TP	FN	FP	TN	Sensitivity	Specificity
Acetest tablet:									
(Nielen et al., 1994)	18	11.3%	124	14	0	45	65	100%	59%
KetoTest:									
(Carrier et al., 2004)	1	18.2%	159	28	1	52	78	97%	60%
Ketostix, ≥ trace (5 μmol/L):									
(Carrier et al., 2004)	1	7.0%	741	47	5	101	588	90%	85%
(Oetzel, 2004)	6	12.0%	83	9	1	18	55	90%	75%
Ketostix, ≥ small (15 μmol/L):									
(Carrier et al., 2004)	1	7.0%	741	41	11	31	658	79%	96%
(Oetzel, 2004)	6	12.0%	83	8	2	6	67	80%	92%
Ketostix, ≥ moderate (40 μmol/L):									
(Carrier et al., 2004)	1	7.0%	741	26	26	7	682	50%	99%
(Oetzel, 2004)	6	12.0%	83	7	3	2	71	70%	97%

BHBA = β-hydroxybutyric acid, mg/dl; Ketosis = blood BHBA ≥ 14.4 mg/dl.
TP = true positives; **FN** = false negatives; **FP** = false positives; **TN** = true negatives.

The best test for cowside urine ketone evaluation is a semi-quantitative dipstick (Ketostix; Bayer Corp. Diagnostics Division, Elkhart, IN) that measures acetoacetate. Urine ketone tests on the whole have a reputation for very poor specificity; however, recent data suggest that poor specificity may not be a problem with the Ketostix. The urine dipstick has good specificity (and sensitivity) compared to the blood BHBA test (Table 4).

Prolonged contact of urine with the reagent may explain some of the false positive results obtained with the urine ketone tablet or the milk BHBA strip. The label for the urine dipsticks states that the test result should be interpreted exactly 15 seconds after contact with the urine sample. Results were read within five seconds in one study (Carrier et al., 2003), and this study reported the highest specificity results for a urine test.

Interestingly, results for urine testing with the Ketostix suggest that lower concentrations (e.g., 'small') should not be ignored if the purpose of the test is to identify cows with ketosis that might benefit from treatment for ketosis. Since oral treatment with glucose precursors is generally inexpensive and safe, it is most appropriate to use a low cut-point for urine ketones in making individual cow treatment decisions. At a cut-point of 'small', only about 2% of urine test negative cows have ketosis, and about 43% of urine test positive cows do not have ketosis (calculated from pooled data presented in Table 4).

Cowside Milk Tests for Ketosis. Cowside milk tests have tremendous advantages over urine cowside tests for ease of collection and for assurance that all eligible cows can be tested. However, milk tests are generally not as sensitive as urine tests in detecting ketosis.

Nitroprusside powders (Utrecht powder, KetoCheck powder) can be used to qualitatively test milk acetoacetate. However, these tests generally have very poor sensitivity for ketosis compared to blood BHBA (see Table 5) and cannot be recommended as tests for herd-based monitoring. They have some, but very limited value as cowside tests for diagnostic decisions for individual cows.

Table 5. Sensitivity and specificity of cowside milk nitroprusside powders compared to blood BHBA (cut-point of ≥11.7 or 14.4 mg/dl).

Test type / Study	BHBA Cut-Point,	Herds Tested	% Ketosis	Total Samples	TP	FN	FP	TN	Sensitivity	Specificity
Utrecht powder:										
(Nielen et al., 1994)	≥14.4	18	10.3%	185	17	2	7	159	89%	96%
(Geishauser et al., 1998)	≥11.7	25	16.4%	529	37	50	0	442	43%	100%
KetoCheck powder (≥ trace):										
(Geishauser et al., 1998)	≥11.7	25	16.4%	529	24	63	0	442	28%	100%
(Carrier et al., 2004)	≥14.4	1	7.5%	878	28	38	9	803	42%	99%
Bioketone powder (≥ trace):										
(Geishauser et al., 1998)	≥11.7	25	16.4%	529	24	63	0	442	28%	100%

BHBA = β-hydroxybutyric acid, mg/dl; **Ketosis** = blood BHBA ≥11.7 or 14.4 mg/dl.
TP = true positives; **FN** = false negatives; **FP** = false positives; **TN** = true negatives.

The most promising cowside milk ketone test is a semi-quantitative milk BHBA test strip manufactured by Sanwa Kagaku Kenkyusho Co., Ltd. (Nagoya, Japan). This test strip is marketed under various names (KetoTest, Ketolac BHBA, and Sanketopaper) in different parts of the world. It is not commercially marketed in the US, although it may be imported into the US from Canada (CDMV, St. Hyacinthe, Quebec) and costs about $2.00 (USD) per strip.

Results of numerous studies evaluating the sensitivity and specificity of the milk BHBA test strip compared to blood BHBA results are presented in Table 6. My own clinical experience with this test (221 cows from 17 herds) corroborates previously published results. When used at the cut-point of ≥100 μmol/L, this test is about 83% sensitive and 82% specific. For individual cow testing, the ≥50 μmol/L cut-point provides better sensitivity (89%) but has a false positive

rate of 69% (calculated from pooled data presented in Table 6). Increasing the cut-point to ≥200 μmol/L reduces test sensitivity to 54% (Table 6). At this higher cut-point the test is of little value for diagnosing ketosis in individual sick cows but has potential use for herd-based evaluations, as discussed later.

Table 7. Sensitivity and specificity of a cowside milk BHBA compared to blood BHBA (cut-point of ≥14.4 mg/dl).

Test type / Study	Herds Tested	% Ketosis	Total Samples	TP	FN	FP	TN	Sensitivity	Specificity
Milk BHBA strip (≥50 μmol/L):									
(Geishauser et al., 2000)	21	11.9%	469	51	5	182	231	91%	56%
(Carrier et al., 2004)	1	7.6%	883	59	8	100	716	88%	88%
(Oetzel, 2004)	17	17.2%	221	34	4	36	147	89%	80%
Pooled data (by cow)	39	10.2%	1573	144	17	318	1094	89%	77%
Milk BHBA strip (≥100 μmol/L):									
(Jorritsma et al., 1998)	8	8.4%	190	14	2	31	143	88%	82%
(Geishauser et al., 2000)	21	11.9%	469	45	11	99	314	80%	76%
(Carrier et al., 2004)	1	16.5%	248	39	2	65	142	95%	69%
(Duffield et al., 2003)	5	27.2%	235	52	12	64	107	81%	63%
(Carrier et al., 2004)	1	7.6%	883	50	17	54	762	75%	93%
(Oetzel, 2004)	17	17.2%	221	33	5	32	151	87%	83%
Pooled data (by cow)	53	12.6%	2246	233	49	345	1619	83%	82%
Milk BHBA strip (≥200 μmol/L):									
(Jorritsma et al., 1998)	8	8.4%	190	14	2	31	143	88%	82%
(Geishauser et al., 2000)	21	11.9%	469	45	11	99	314	80%	76%
(Duffield et al., 2003)	5	27.2%	235	52	12	64	107	81%	63%
(Carrier et al., 2004)	1	7.6%	883	50	17	54	762	75%	93%
(Oetzel, 2004)	17	17.2%	221	17	21	5	178	45%	97%
Pooled data (by cow)	52	12.1%	1998	129	112	100	1657	54%	94%

BHBA = β-hydroxybutyric acid, mg/d/L; **Ketosis** = blood BHBA ≥14.4 mg/dl.
TP = true positives; **FN** = false negatives; **FP** = false positives; **TN** = true negatives.

The cowside milk BHBA test strip has limited value for herd-based monitoring of ketosis. Blood BHBA test results are much more reliable for this purpose, and immediate cowside results are not particularly critical for herd-based testing (as they are for individual sick cow diagnosis). The imperfect sensitivity and specificity of the milk BHBA test distort the prevalence of ketosis in a herd. The true herd prevalence of ketosis may be either higher or lower than the prevalence measured by the milk BHBA test strip, depending on the cut-point chosen (Table 7). The degree

of disparity between ketosis prevalence determined by the milk BHBA strip vs. the blood BHBA test also depends on the true prevalence of ketosis. The best cut-point for herd monitoring when using the milk BHBA strip appears to be ≥200 μmol/L. At this cut-point the prevalence of test positive results is similar to the true prevalence, allowing the same alarm level for ketosis prevalence (10%) to be used for both tests. Unfortunately, milk BHBA test strip prevalence changes little as true prevalence increases (Table 7), rendering the test practically useful only for identifying herds with a very high prevalence of ketosis.

Table 7. Expected test positive prevalences for milk BHBA test strip results at different true herd prevalences and test strip cut-points.

	Herd Ketosis Prevalence Category:			
	Low	**Alarm Level**	**Moderate**	**High**
Milk BHBA ≥ 50 μmol/L (89% sensitivity, 77% specificity):				
True prevalence	7.5%	10.0%	15.0%	30.0%
Milk strip test positive prevalence	28.0%	29.6%	32.9%	42.8%
Milk BHBA ≥ 100 μmol/L (83% sensitivity, 82% specificity):				
True prevalence	7.5%	10.0%	15.0%	30.0%
Milk strip test positive prevalence	22.9%	24.5%	27.8%	37.5%
Milk BHBA ≥ 200 μmol/L (54% sensitivity, 94% specificity):				
True prevalence	7.5%	10.0%	15.0%	30.0%
Milk strip test positive prevalence	9.6%	10.8%	13.2%	20.4%

BHBA = β-hydroxybutyric acid; **Ketosis** = blood BHBA ≥14.4 mg/dl.

Results from my herd investigations illustrate the difficulty in using the milk BHBA test strip for herd-based monitoring. In nine herds I had sufficient sample size to categorize the herd for ketosis using both milk BHBA (≥200 μmol/L cut-point, 10% alarm level) and blood BHBA (≥14.4 mg/dl cut-point, 10% alarm level). Categorization of five herds was the same using either test method. However, two herds classified positive by blood BHBA were classified negative by the milk BHBA test strip, and both herds had apparently high ketosis prevalences (44% and 24%). The classification of two other herds was different for the milk BHBA test strip compared to the blood BHBA test.

Cowside Blood Tests for Ketosis. We are in the process of evaluating a cowside blood BHBA test system using a human instrument marketed for diabetic patients (Precision Xtra™ blood glucose and ketone monitoring system, Abbott Laboratories, Abbott Park, IL). This metering system uses a small hand-held meter (retail cost of about $70 USD, although it is often on sale for less than $10 USD). The meter can measure either whole blood glucose or whole blood BHBA. Glucose test strips cost about $0.60 USD each, and ketone (BHBA) test strips cost about $4.00 USD each. The glucometer / ketometer system is very easy to use cowside. A strip is inserted in the meter, less than a drop of blood is added to end of the strip, and results are

displayed in about 15 seconds. The strips do not require calibration prior to use. We are using the system without modification, with the existing human calibrations.

We collect a small amount of blood from the tail vein using a small needle (25 gauge) and small syringe (1 ml) to use with the glucometer / ketometer. It is easy to collect a very small volume of blood (<0.1 ml) in the syringe. We then apply a small drop of this blood to the test strip. The lancets provided with the system for human use do not work for cows, even when we try them on thin skin in the tail fold.

Our preliminary results with the glucometer / ketometer system are very encouraging. We have paired hundreds of serum and plasma samples sent to a commercial laboratory with cowside test results on whole blood. The system is more accurate as a ketometer (for BHBA) than as a glucometer (glucose). Sensitivity and specificity for BHBA appear to be outstanding (over 95%).

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Appendix I - Silage Fermentation Analyses

Silage Fermentation Process. Proper silage fermentation starts with forage harvested at the proper dry matter content. This silage should then be chopped to the proper particle length. Finer chopping tends to improve packing and silage fermentation products, but conversely increases the risk for ruminal acidosis. So, proper chop length represents a compromise between maximal silo packing and optimal rumen health.

Once silage has been harvested at the proper dry matter content and chopped to the correct particle length, it must be packed as quickly and tightly as possible in the silo. The goal during silo filling is exclusion of as much air as possible so that the silage goes through only a short aerobic stage of fermentation. This stage of fermentation ends when aerobic bacteria consume the oxygen in the silage. Heat is a major end product of aerobic fermentation, so it is desirable to limit it as much as possible. With good silage, this phase only lasts a few days and the silage does not become excessively hot.

The aerobic phase of silage fermentation will be extended if the silage is too dry when chopped, loosely packed in the silo, or if the top layer of the silo is exposed to excessive oxygen because of slow filling. Any of these problems leads to excessive loss of silage dry matter as soluble carbohydrates are converted to heat and lost. Additionally, excessive heating may damage silage proteins by irreversibly binding them to fiber and making them indigestible. Heat-damaged proteins can be measured as bound protein (also termed ADF-CP, ADF-N, ADIN, unavailable protein, or Maillard protein).

Once the oxygen in the silage is consumed, fermentation moves into the anaerobic phase. Anaerobic bacteria initially convert soluble carbohydrates in the silage into acetic acid. As the pH of the silage drops from the accumulation of acetic acid, lactic-acid producing bacteria dominate. These bacteria produce lactic acid until the pH of the silage drops below about 4 or 5. At that point, all bacteria in the silage die and the silage becomes a stable, preserved mass. The anaerobic phase of silage fermentation takes about 1 to 3 weeks, depending on the availability of soluble carbohydrates in the ensiled material and the ambient temperature (fermentation proceeds slower during cooler weather). Anaerobic fermentation does not produce heat or cause loss of silage dry matter.

Silage proteins are partially degraded to ammonia and other forms of non-protein nitrogen during aerobic and anaerobic fermentation. Excessive protein degradation is undesirable and reduces the nutritional value of the forage. The lower the dry matter of the ensiled material, the more extensive the protein degradation. Proper forage dry matter at ensiling is a compromise between rapid oxygen exclusion and risk of improper silage fermentation at very low dry matters. Wetter forages have advantages of more rapid oxygen exclusion and rapid acid production, but they suffer from extensive protein degradation. Wetter forages may also favor fermentation by clostridial species of bacteria, which produce very undesirable fermentation end products such as butyric acid and toxic protein amines. Clostridial fermentation does not lower silage pH below 5.0 and thus is not self-limiting. Butyric acid concentrations in silages fermented by clostridial bacteria continue to rise during storage as long as the silage pH stays above about 5.0.

When the silage is fed out of the silo, it is re-exposed to oxygen and undergoes a secondary aerobic fermentation. Heat is produced during this phase, plus spoilage bacteria, yeasts, and molds may grow as silage pH rises. Silages with a lower final pH and higher total acid content are less vulnerable to secondary fermentation problems than are poorly fermented silages with higher final pH's and lower total acid content. Acetic acid is more effective in inhibiting secondary fermentation than is lactic acid; however, high acetic acid content may inhibit feed intake and is not desirable. Well-fermented silages typically contain about twice as much lactic acid as acetic acid.

Silage Fermentation Analysis. Silage pH can be determined on the farm. Add about 1 tablespoon of silage in about 50 ml distilled or deionized water and mix well. Then determine pH using a pH meter (Cardy Twin pH Meter, (Spectrum Technologies, Plainfield, IL, 800-248-8873 or http://www.specmeters.com). or pH paper strips (Whatman Lab, pH indicator paper range 4 to 6, type CS, 800/942-9626). Laboratories can perform a full silage fermentation (VFA) analysis that typically includes silage dry matter, pH, crude protein, lactic acid, acetic acid, propionic acid, butyric acid, iso-butyric acid, total acids, ethanol, and ammonia. A hypothetical example of a report from a silage fermentation analysis is listed on the next page.

S A M P L E   F E R M E N T A T I O N   A N A L Y S I S

Test Item	Value	Unit	Normal Range (alf. silage)
Dry Matter	30.3	% DM	35 – 45
Crude Protein	22.6	% DM	18 - 22
Bound Protein	1.0	% DM	.3 – 2.2
Bound Protein	4.4	% CP	2 - 10
Ammonia (CP equivalent)	5.0	% DM	1 – 2.5
Ammonia (CP equivalent)	22.2	% CP	7 - 15
pH	5.43		4.2 – 4.8
Lactic acid	1.7	% DM	2 – 4
Acetic acid	4.63	% DM	1 – 2
Propionic acid	0.59	% DM	0 - .25
Iso-butyric acid	0.22	% DM	<.01
Butyric acid	2.24	% DM	<.01
Total fermentation acids	9.38	% DM	3 – 6
Lactic acid/total acids	18.12	% acids	> 50
Ethanol	0.0	% DM	<.01

Results of this analysis show a typical pattern of an overly wet alfalfa silage that has gone through a mixed fermentation pattern that includes clostridial bacteria. The end result is a silage that is high in ammonia, high in pH, low in lactic acid, and high in butyric and acetic acids.

Expected values from silage fermentation analysis for alfalfa silage, grass silage, corn silage, and high moisture shelled corn are presented in Appendix II.

Appendix II. Normal Values for Ensiled Feed Fermentation Analyses

Test Item	Alfalfa or Grass Silage	Corn Silage	Hi Moist. Sh. Corn
Dry matter, %	35 – 45	30 – 35	65 – 75
Crude protein, % DM	18 – 22	7 – 9	8 – 10
Bound protein, % CP	2 – 10	2 – 8	2 – 8
Ammonia (CP equiv), % DM	1.0 – 2.5	.2 – 1.0	---
Ammonia (CP equiv), % CP	7 – 15	5 – 10	---
pH	4.2 – 4.8	3.5 – 3.8	4.2 – 4.8
Lactic acid, % DM	2 – 4	3 – 6	---
Acetic acid, % DM	1 – 2	1 – 3	---
Propionic acid, % DM	0 - .25	0 - .25	---
Iso-butyric acid, % DM	<.01	<.01	<.01
Butyric acid, % DM	<.01	<.01	<.01
Total acids, % DM	3 – 6	4 – 9	---
Lactic acid, % total acids	>50	>60	---
Ethanol, % DM	<.01	<.01	<.01

Appendix III - Dietary butyric acid and ketosis: A summary of published research trials

Trial	Butyric acid source	Butyric acid in the silage	Butyric acid daily dose	Experimental animals	Result
Tveit B, et al., J Dairy Sci 75:2421, 1992 (Norway)	"Dark bunker" silage – smelled bad and averaged 20% dry matter. The same bunker was sampled 4 times during the study.	Average was .55% butyric acid (DM basis), highest sample was about 1.85% butyric acid (DM basis)	The authors estimated 30 kg/day of as-fed silage intake; therefore, the average butyric acid intake was about 33 g/day. The highest expected intake was about 111 g/day.	12 total cows; stage of lactation not specified	Blood AcAc increased from about 100 umol/l to 250 - 400 umol/l when cows were fed the dark bunker silage. Ketosis is defined as >360 umol/l blood AcAc, so these cows were ketotic. Values reported were group means, so some individual cows had much higher AcAc.
Schultz LH, J Dairy Sci 54:962, 1971 (Wisconsin)	High moisture hay crop silage (probably alfalfa or alfalfa-grass mix - the paper does not state what kind of hay crop it was).	3.7% butyric acid (probably on a dry matter basis, although this is not specified)	The amount of silage fed was not specified. At 20 lbs of forage DM intake, the daily butyric acid dose would be 366 g/cow/day.	Number of cows not specified. They were not in early lactation and thus were beyond their highest risk for ketosis.	Total blood ketones were raised from 4 mg/dl (control cows, low moisture forage) to 22 mg/dl. Total blood ketones include AcAc plus BHBA. This level of total blood ketones represents ketosis. These cows might have had much higher blood ketones if they had been in early lactation.
Andersson L and Lundstrom K, Zentralbl. Vet. Reihe A 32:15, 1985 (Germany)	Silage high in butyric acid. This is a German language article; secondary reference from Tveit, et al.	Not specified	Up to and over 100 g/cow/day	Not specified	Milk ketone concentrations tended to increase when silage butyric acid intake exceeded 100 g/day (as quoted from Tveit, et al.).
Lingass F and Tveit B, J Dairy Sci 75:2433, 1992 (Norway)	Oral dosing with butyric acid via a ruminal tube	---	200 g butyric acid per day	Eight cows in early lactation	Blood AcAc increased to an average of about 525 umol/l after dosing; these are very high levels. Since this is an average value, many of the individual cows had much higher blood AcAc concentrations. Eight later lactation cows were also tested; but their blood AcAc concentrations were only about 50% as high.
Dirksen G. and Breitner W., J. Vet. Med. A 40:779-784, 1993 (Germany)	Intra-ruminal infusion of butyric acid	---	400 ml butyric acid. The density of butyric acid is .958 g/ml, so this equals 383 g of actual butyric acid.	Three lactating cows, low production (25 to 35 lbs milk/cow/day)	Blood BHBA increased rapidly to about 3500 umol/l, which is very high. The study does not mention whether or not these cows showed clinical signs of ketosis, but they would be expected to show clinical signs at these very high blood BHBA's.
Mills SE, Beitz DC, Young JW, J Dairy Sci 69:352, 1986 (Iowa)	A ketogenic substrate (1,3 butanediol) that is not metabolized in the rumen was fed. Cows were given 500 to 1000 g/cow/day to induce clinical ketosis.	Cows were also offered less feed (80 to 85% of previous ad libitum intake) to help induce ketosis.	A dose of 500 g of 1,3 butanediol (90.12 g/mole) provides the same potential moles of BHBA as 489 g of butyric acid (88.11 g/mole). So, the equivalent dose of butyric acid was 489 to 978 g/cow/day.	Five high-producing cows. Ketosis induction was started 2 weeks after calving.	Blood BHBA concentrations increased to 3100 to 4500 umol/l. These are extremely high blood BHBA concentrations. Four of five cows showed clinical signs of ketosis. Only one cow (a lower producer) did not develop clinical signs of ketosis. The authors considered the ketosis induction protocol to be successful.