

Interpretation of Serum Metabolic Parameters Around the Transition Period

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SUMMARY

- The two most useful metabolic tests for transition cows are precalving serum non esterified fatty acids (NEFA) and postcalving beta-hydroxybutyrate (BHBA).
- Elevations in these parameters are associated with increased clinical disease risk, reduced reproductive performance, impaired milk yield, and increased risk of culling.
- These tests are best used from a herd diagnostic perspective with high prevalence of high NEFA or high BHBA suggesting that investigation of transition cow management and nutrition is warranted.
- Metabolic tests should be used as one component of an investigation and not as the sole measure of identifying a problem.

INTRODUCTION

Metabolic profiling has its roots in Compton, England in the 1970's. Since that time automated laboratory machines and enzyme kits to measure a battery of serum or blood constituents is widespread and commonplace. However, ease of sample collection and submission is not justification for conducting metabolic herd testing. Serum analytes have more value when used appropriately in the diagnostic process or as part of a specific objective in herd monitoring programs for metabolic disease. Profile testing should not be used in place of other more appropriate procedures, such as ration evaluation and physical examinations. The transition cow represents the target with the most utility for metabolic profiling.

Most periparturient abnormalities have some metabolic element as a component of the sufficient cause of clinical disease. Excess negative energy balance, fat mobilization and subsequent elevations in ketone body concentrations play a contributing role in the expression fatty liver syndrome, clinical ketosis, and abomasal displacement. A severe negative energy balance during transition may also increase the risk of retained placenta, metritis, and mastitis through impaired immune function. In addition to energy balance, nitrogen balance and calcium homeostasis are disrupted through parturition. Therefore, several biochemical parameters may be useful for monitoring cows in the transition period.

OBJECTIVES OF METABOLIC PROFILING

There can be two main objectives for conducting serum metabolite testing in periparturient cows. Although these objectives may overlap, it is worth stating them for clarity.

1. Cow-level interpretation – There is a problem with this cow and treatment and/or further examination may be warranted.

2. Herd-level interpretation - There is a potential problem with the current herd management that needs to be investigated.

Cow and herd level interpretation can be conducted with the same samples but they differ in that we are differentiating between an individual or group problem. In our opinion the group interpretation is the strongest reason for conducting the tests regardless of whether it is an ongoing monitoring program or a herd-problem investigation. Cow level interpretation of abnormal results may suggest a higher health and production risk for that cow but does not necessarily indicate the cow is a wreck. Also, little is known about the benefits of treating individual cows with high metabolic parameters. Group level measures that are high suggest that there might be management and/or nutritional issues worth investigating.

SERUM METABOLITES TO CONSIDER

Circulating concentrations of non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) measure the success of adaptation to negative energy balance. NEFA reflects the magnitude of mobilization of fat from storage. BHBA indicates the completeness of oxidation (“burning”) of fat in the liver. Ketone bodies (BHBA, acetone and acetoacetate) are intermediate metabolites of oxidation of fatty acids; as the supply of NEFA to the liver exceeds the ability of liver to completely oxidize the fatty acids to supply energy, the amount of ketone body production increases. Ketone bodies can be used by muscle as an alternative fuel source to glucose, sparing glucose for milk production (Herdt, 2000a). However, ketone production does not result in as much net energy release as does complete oxidation of fatty acids. Additionally, increasing concentrations of ketones are thought to suppress feed intake.

Glucose is the primary metabolic fuel, and is absolutely required for vital organ function, fetal growth, and milk production. In dairy cows, the massive energy demand to support milk production is partly met through gluconeogenesis. Glucose concentrations are under tight homeostatic control. Therefore, although glucose has a central role in metabolism, it is a poor analyte for monitoring or investigating herd problems (Herdt, 2000b). Aspartate aminotransferase (AST) is an enzyme that becomes elevated with cell damage and may be elevated in cows with fatty liver disease. Although there have been associations between AST and subsequent occurrence of displaced abomasums (Geishauser et al, 1997), the test lacks both sensitivity and specificity. For assessing the adaptive response to energy balance NEFA and BHBA are the best two measures.

Calcium demand is tremendous immediately postpartum and monitoring serum calcium in cows less than a week following calving may have some utility but before or beyond this time period, it makes no sense to measure calcium. Recently, low serum calcium concentrations (subclinical hypocalcemia) have been linked with increased risk of early lactation culling (Duffield et al, 2005).

Haptoglobin is an acute phase protein that becomes elevated under situations of inflammation. However, this inflammation indicator is non-specific and could reflect for example dystocia, mastitis, metritis or displaced abomasum. However, despite its non-specific nature haptoglobin may have utility for monitoring transition cows. Recently, haptoglobin has been used shortly following calving as an indicator of pending clinical metritis (Huzzey et al, 2009).

Currently the strongest data exists for the use of NEFA and BHBA testing in transition dairy cows. The remainder of this article will focus on these two analytes.

Key associations of NEFA and BHBA with health and performance in transition dairy cows are:

- High NEFA (> 0.4 mmol/L) in the 2 weeks before calving is associated with
 - o 2 to 4 times increased risk of LDA (Cameron et al, 1998; LeBlanc et al, 2005)
 - o 1.8 times increased risk of retained placenta (RP) (LeBlanc et al 2004)
 - o 2 times increased of culling before 60 days in milk (DIM) and 1.5 times increased risk of culling over the whole lactation (Duffield et al, 2005)
 - o 1.2 kg/day milk production loss for the 1st 120 days of lactation (Carson, 2008)
- Subclinical ketosis (BHBA $> 1200 - 1400$ $\mu\text{mol/L}$) in early lactation is associated with
 - o 3 to 8 times increased risk of LDA (; Geishauser et al, 2000b; LeBlanc et al 2005; Duffield et al, 2009)
 - o Decreased probability of pregnancy at first AI (Walsh et al, 2007)
 - o Decreased milk production (Duffield et al, 2009)
 - o Increased duration and severity of mastitis (Suriyasathaporn, 2000)

MONITORING NEFA and BHBA

Cow Selection

By most definitions, the theoretical testing period for transition cows would extend from 3 weeks prior to calving until 3 weeks after calving. Practically however, the most important time periods are:

1. During the last week prior to calving.
2. Within the first 2 weeks after calving.

Precalving

It is unusual for cows to develop subclinical ketosis (SCK) precalving because the etiology of the condition depends on the homeorhetic drive for milk production. However, cows will start mobilizing energy reserves in the final week before parturition. This can be measured via serum or plasma NEFA. The challenge for this precalving sample is predicting when the animal is going to calve. In the past, establishment of a serum bank and retrospective submission of samples relative to calving have been recommended. However, recent data suggests that assessment of samples obtained within a week of expected calving is a practical approach that seems to provide meaningful information (Leblanc et al, 2005).

Postcalving

A routine ketone testing program should commence after calving. The primary risk period for SCK is the first month of calving. The first 2 weeks postcalving is the time of peak incidence. In addition, the median days from calving to diagnosis of clinical ketosis and displaced abomasum is around 11 days. Thus, in order to try to prevent subclinical disease from becoming clinical disease (if that is possible), cows must be identified earlier. For these reasons, a SCK monitoring program should focus on the first 2 weeks of lactation. Recent work

has suggested that elevated BHBA in the first week postcalving has more impact on both cow health and milk yield reduction than elevations later in lactation (Duffield et al, 2009). Although elevated NEFA in week 1 and 2 postcalving was associated with an increased risk of abomasal displacement, these cows produced more milk (Carson, 2008). It is worth noting that mobilization of fat and partial oxidation to ketones is part of the adaptive response to negative energy balance and supports milk production. Thus the goal should be to limit the negative effects of a poor adaptive response. There is likely variation in individual cows capacity to tolerate higher NEFA and BHBA, therefore interpretation of individual cow tests exclusive of other signs may not always be useful. However, having a high proportion of a group with high NEFA and/or BHBA is meaningful and would be more likely to result in increased disease incidence and production loss.

Required Sample Size

The number available for testing depends on the herd size. For both BHBA and NEFA it is proportion rather than mean measurements that are important. A good thumb rule for evaluating a herd is to interpret data based on 12 samples. This is based on the following: in a close-up or fresh cow group of up to 500 cows, assuming that detection of a prevalence of subclinical disease of 10% is the threshold of interest, to have 75% confidence of detecting the problem, 13 samples are required (Dohoo et al, 2003). Oetzel (2004) proposes using 12 samples for simplicity of interpretation. In small herds, this may require repeated sampling over time.

TEST SELECTION

NEFA

This test should only be used precalving on samples obtained within 1 week of parturition. The data for NEFA is frequently right skewed and thus averages can be very misleading. One suggested threshold is 0.5 units/L. In recent work, cows within 1 week of calving with serum NEFA above this threshold were at a 3.5 times greater risk of subsequently developing a displaced abomasums (Leblanc et al, 2005). Whole herd interpretation is best made by calculating a proportion of cows above a threshold value, however, there is limited data on an appropriate goal for this parameter. In a multi-herd 1060 cow study near Guelph, 30% of cows were above 0.5 U/L during the last week prior to calving (Leblanc et al, 2005).

The potential of precalving NEFA as a monitoring tool is further highlighted by research conducted at the Elora dairy research center (Osborne, 2003). Of 136 transition cows evaluated, 24 had BHBA concentrations $\geq 1400 \mu\text{mol/L}$ of serum in the first week post-calving (17.6%). There was a significant association between NEFA concentration in the week prior to calving and BHBA concentration in the first week post-calving. A nearly 5-fold increased risk of SCK was noted when the NEFA concentrations in the week before calving were greater than 0.7 mmol/L ($OR=4.8$, $P=0.04$) (Osborne, 2003).

BHBA

In contrast to NEFA, serum BHBA should only be used postcalving. The first two weeks are the primary risk period for subclinical ketosis, defined by a serum concentration of 1400 $\mu\text{mol/L}$ BHBA or greater (Duffield, 2000). A reasonable goal is to have less than 2 cows per 10 with BHBA above 1400 $\mu\text{mol/L}$ in the first 2 weeks post-calving.

Sample Handling

Both NEFA and BHBA can be measured with either plasma or serum. Both analytes are subject to interference with hemoglobin in the sample, thus, hemolysis will artificially elevate measurements and should be avoided. Both NEFA and BHBA are subject to changes relative to time of feeding. Samples meant to compare performance on the same farm should be obtained at approximately the same time of day. The most severe swing in values in our experience appears to be with NEFA with highest values obtained just before first feeding. Therefore it is best to sample herds at some point after the first feeding of the day. NEFA concentrations could be slightly falsely elevated if serum were not separated within 12-24 h of blood collection, or if samples were not kept chilled (Stokol and Nydam, 2004). Serum can be kept frozen for at least 1 month without affecting NEFA results. Samples should be collected from the tail vein (not the milk vein) and ideally chilled, separated within a few hours, and then frozen or shipped chilled for receipt at the laboratory within 1 to 2 days. However, delay of up to 24 hours for separation, and kept at room temperature for 1 day or refrigerated for < 3 days does not substantially affect results (Stokol and Nydam, 2004).

INTERPRETATION OF NEFA AND BHBA RESULTS

Suppose you have just tested 40 close-up cows within 1 week from expected calving and find that 10 cows have NEFA values at or above 0.5 mmol/L, what do you do with the results? First, calculate the prevalence of high NEFA cows: $10/40 \times 100 = 25\%$. Is this a problem herd for high precalving NEFA? Some would argue that it likely is, however recent data from a study involving 55 herds across the U.S. and Canada, suggest that this is probably average. In the 55 herd dataset, the median prevalence of high precalving NEFA (≥ 0.5 mmol/L) was 25%, with a minimum of 6.5% and a maximum of 75%. However when herds were classified into high and low risk using the median of 25% as the cutoff, the high risk herds tended to have less milk production, lower milk protein, and were significantly more likely to have a higher incidence of displaced abomasum (See Table 1 below). Thus as a starting point we suggest that a reasonable goal is for herds to have less than 25% of cows with prefresh NEFA at or above 0.5 mmol/L. Herds with prevalence values higher than this can be classified as high risk but are not necessarily problem herds.

From the same data, the median prevalence of high BHBA (≥ 1400 umol/L) for week 1 after calving was 15% with a minimum of 0 and a maximum of 45%. As a starting point we suggest 15% for week 1 following calving be used to distinguish high risk from low risk herds for high BHBA.

Table 1. Cow Health and Production Parameters associated with Herd Level Precalving NEFA status

	Low Risk Herd Proportion of Precalving High NEFA (≥ 0.5 mmol/L) cows < 25% N=26	High Risk Herd Proportion of Precalving High NEFA (≥ 0.5 mmol/L) cows $\geq 25\%$ N=29
Displaced Abomasum Incidence (DA)	3.6%	4.7%
Proportion of Herds with DA incidence > 3.3%	38%	65%
Milk Production for 1 st 120 days (kg/d)	39.5	39.3
Proportion of Herds with milk production > 39.6	58%	27%
1 st DHI test Protein	3.07	2.97
Proportion with 1 st Test Milk Protein > 3.00	60%	37.5%

COWSIDE TESTS

Milk Ketone Tests

Most milk ketone tests measure acetone and acetoacetate through a reaction with nitroprusside which causes a colour change from white to pink or purple. These tests in general are poorly sensitive in milk (<40%) but highly specific (>90%) (Duffield, 1997; Geishauser et al, 1998). One exception is a milk ketone test that measures BHBA. It is marketed in Europe as “Ketolac BHB”, in Japan as “Sanketopaper”, and in Canada as “Keto-Test”. This test has a much higher sensitivity in milk (>70%) and reasonably good specificity (>70%, up to 90%) (Oetzel, 2004). This is a semi-quantitative test that allows choosing a lower threshold for screening to increase sensitivity, and a higher threshold for diagnosis to increase specificity.

Urine Ketone Tests

The urine ketone tablet tests are based on the same nitroprusside reaction as the milk powder ketone tests. These tests are highly sensitive (approaching 100%) but are poorly specific. Thus, they are great tests for ruling out subclinical ketosis with a negative test result. However, their use overestimates a subclinical ketosis problem because of a high probability of false positive reactions (see table 1). However, recent work out of Minnesota suggests that a 5 to 10 second interpretation using the Ketostix in urine is just as accurate as the Keto-Test in milk (Carrier et al, 2005).

Serum Tests

Several research abstracts have been published lately on the use of the Precision Xtra glucometer measuring ketones (which are beta-hydroxybutyrate). The studies have shown repeatedly high correlation with laboratory measures for BHBA.

Selection and Interpretation of Cowside Tests

There are two possible actions resulting from screening a group of fresh cows with a ketone test. One action might be to treat all positive animals with the goal to prevent subsequent development of clinical disease. In this case, a high predictive value of a positive test is desired so that normal animals are not unnecessarily treated. The second action might be to compare the percent of positive reactors to a goal for determining the effectiveness of either the transition ration or some prophylactic measure in reducing the incidence of subclinical ketosis. In this situation, the apparent prevalence is the parameter that actually would be used. Note from Table 2 that the urine Acetest tablet would substantially overestimate the prevalence of subclinical ketosis, while the Ketocheck™ test would grossly underestimate the prevalence. Despite a consideration of the inherent sensitivity and specificity of these two tests, their utility for group level decision making is questionable. The Acetest might be used with an adjustment in the apparent prevalence goal. The Ketocheck test is simply too insensitive to be useful. However, both the Keto-Test and the Ketostix are useful tests for group level monitoring and for individual animal identification.

Table 2. Use of Cowside Ketone Tests in Screening Programs for Identifying Subclinical Ketosis.

Test	20% Prevalence			40% Prevalence			60% Prevalence		
	PV +ve	PV -ve	Apparent Prevalence	PV +ve	PV -ve	Apparent Prevalence	PV +ve	PV -ve	Apparent Prevalence
Keto-Test® using 100 µmol/L	62%	93%		81%	83%		91%	68%	
Ketochek™ (milk)	90%	86%	8%	96%	70%	16%	98%	51%	23%
Urine Acetest Tablet	38%	100%	53%	62%	100%	65%	78%	100%	76%
Urine Ketostix	83%	94%	19%	93%	87%	34%	97%	74%	48%

PV +ve: Predictive Value of a positive test result. PV –ve: Predictive Value of a negative test result.

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