Peripartum Metabolic Monitoring

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Abstract

Peripartum metabolic monitoring for the lactating dairy cow is best directed at measures of adaptation to negative energy balance. Use of serum non-esterified fatty acids (NEFA) prepartum and a measure of ketone concentrations postpartum currently offer the best window into transition cow energy status. These tests can be directed at a herd problem investigation or used in a routine monitoring program. Routine monitoring of prepartum dairy cows should be conducted using serum NEFA. This currently poses several limitations, including cost, for many dairy farms. A regular monitoring program for measuring ketones can fit into an existing fresh cow monitoring system. Cowside ketone tests using either milk beta-hydroxybutyrate or urine acetone/acetoacetate tests offer adequate test performance at a reasonable cost compared to laboratory evaluation of blood or serum. Generally, the most useful information gained from these programs is identification and investigation of herd-level issues rather than diagnosis and treatment of individual animals.

Résumé

La meilleure orientation de la surveillance métabolique péri-partum de la vache laitière en lactation doit cibler des mesures d'adaptation à un bilan énergétique négatif. L'utilisation pré-partum d'acides gras non estérifiés (AGNE) sériques et une mesure post-partum des concentrations des corps cétoniques offrent actuellement la meilleure voie d'observation de l'état énergétique des vaches en transition. De tels tests peuvent servir aux recherches sur les problèmes des troupeaux ou être utilisés dans un programme régulier de surveillance. La surveillance courante des vaches laitières avant la mise bas doit être réalisée en utilisant des AGNE sériques. Pour plusieurs fermes laitières, cela n'est pas possible pour plusieurs raisons dont les coûts associés. Un programme régulier de surveillance associé à la mesure des corps cétoniques peut s'insérer dans un système existant de surveillance de vaches en début de lactation. La mesure des corps cétoniques effectuée en utilisant des tests rapides à la ferme soit sur le bêta-hydroxybutyrate du lait ou l'acétone/acétoacétate de l'urine présente une performance adéquate à un coût raisonnable, comparativement à l'évaluation en laboratoire du sang ou du sérum. En règle générale, l'information la plus utile obtenue de ces programmes est la détermination et la recherche des enjeux au niveau des troupeaux, plutôt que le diagnostic et le traitement individuel d'animaux.

Introduction

Metabolic monitoring has its roots in metabolic profiling developed in Compton, England in the 1970s. 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. Negative energy balance, fat mobilization and subsequent elevations in ketone body concentrations play a contributing role in the expression of fatty liver syndrome, clinical ketosis and abomasal displacement. A 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 Monitoring

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 ex-

amination may be warranted.
2. Herd-level interpretation - there is a potential problem with current herd management that needs to be investigated.

Cow and herd-level interpretation can be conducted with the same samples, but differ in that we are distinguishing between an individual or group problem. In our opinion, group interpretation is the strongest reason for conducting the tests, regardless of whether it is an ongoing monitoring program or a herd-problem investigation.

Serum Metabolites to Consider

Circulating concentrations of non-esterified fatty acid (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 oxidization ("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.[12] 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.[13]

Aspartate aminotransferase 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[11], the test lacks both sensitivity and specificity. For energy balance, NEFA and BHBA are the best two measures.

Calcium demand is tremendous immediately postpartum. 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.[6]

Haptoglobin is an acute-phase protein that becomes elevated under situations of inflammation. However, this inflammation indicator is non-specific and could reflect other problems such as dystocia, mastitis, metritis or displaced abomasum. However, despite its non-specific nature haptoglobin may have utility for monitoring transition cows. Since the strongest data currently 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 in the two weeks before calving is associated with:
  - two to four times increased risk of left-displaced abomasums (LDA);[1,14]
  - 1.8 times increased risk of retained placenta (RP);[14]
  - two times increased likelihood of culling before 60 days-in-milk (DIM) and 1.5 times increased risk of culling over the whole lactation.[6]
- Subclinical ketosis (BHBA > 1200-1400 μmol/L) in early lactation is associated with:
  - three to eight times increased risk of LDA;[5,11,14]
  - decreased probability of pregnancy at first artificial insemination (AI);[19]
  - decreased milk production;[5]
  - increased duration and severity of mastitis.[18]

Monitoring NEFA and BHBA

Cow selection

By most definitions, the theoretical testing period for transition cows would extend from three weeks prepartum until three weeks postpartum. Practically, however, the most important time periods are:

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

Precalving

It is unusual for cows to develop subclinical ketosis (SCK) pre-calving because the etiology of the condition depends on the homeorhetic drive for milk production. However, cows in a precalving energy deficit 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.[14]

Postcalving

A routine ketone testing program should commence after calving. The primary risk period for SCK is the first month of calving. The first two weeks postpartum is the time of peak incidence. In addition, median time period 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 two to three weeks of lactation.

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.[7] Oetzel proposes using 12 samples for simplicity of interpretation.[15] In small herds, this may require repeated sampling over time.

Test Selection

NEFA

This test should only be used prepartum on samples obtained within one week of parturition. 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 one week of calving with serum NEFA above this threshold were at a 3.5 times greater risk of subsequently developing a displaced abomasum.[14] 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 muti-herd, 1060 cow study near Guelph, 30% of cows were above 0.5 U/L during the last week prior to calving.[14]

The potential of NEFA as a monitoring tool is further highlighted by research conducted at the Elora Dairy Research Center.[16] Of 136 transition cows evaluated, 24 had BHBA concentrations ≥ 1400 μmol/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 fivefold 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).[16]

BHBA

In contrast to NEFA, serum BHBA should only be used post-calving. The first two weeks are the primary risk period for subclinical ketosis, defined by a serum concentration of 1400 umol/L BHBA or greater.[3] A reasonable goal is to have less than two cows per 10 with BHBA above 1400 umol/L in the first two 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 hours of blood collection, or if samples were not kept chilled.[17] Serum can be kept frozen for at least one 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 one to two days. However, delay of up to 24 hours for separation, and kept at room temperature for one day or refrigerated for < 3 days does not substantially affect results.[17]

Cowside Tests

Milk ketone tests

Most milk ketone tests measure acetone and acetoacetate through a reaction with nitroprusside, which causes a color change from white to pink or purple. These tests in general are poorly sensitive in milk (<40%) but highly specific (>90%).[5, 10] One exception is the 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%).[15] 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 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 (Table 1). However, recent work out of Minnesota suggests that a five to ten-second interpretation using the Ketostix in urine is just as accurate as the Keto-Test in milk.[2]

Selection and interpretation of cowside tests

Two possible actions can result 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 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 1 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.

Other Tests

Herd disease records

Herd records are important tools for monitoring the incidence of periparturient disease. However, it is highly critical that standardized disease definitions are in place to allow comparison from year to year and from farm to farm. Producers should set goals for minimizing the incidence of metabolic disease. Herd consultants should periodically review herd performance relative to these goals. In addition, intervention levels should be considered. Several diseases are associated with increasing age, and this must be taken into account when assessing herd performance. For example, in monitoring and comparing herd incidence of milk fever and clinical ketosis, it is important to stratify this by parity. A high proportion of first-lactation animals will likely give a herd a much lower incidence of milk fever and clinical ketosis, since risk increases with age.

Dry matter intake

Clearly, cows that are mobilizing NEFA pre-calving will have sub-optimal dry matter intake. In a recently completed project, serum BHBA concentration in the first week post-calving was significantly associated with the average DMI in the week prior to calving .[16] There was a significant increase in the risk of subclinical ketosis (BHBA ≥ 1400 μmol/L of blood serum) if the DMI was below 12 kg/day (OR=5.7, P=0.05) in the three weeks prior to calving. If the DMI in the week prior to calving was below 11 kg/day, there was a greater risk of an animal developing subclinical ketosis in the first or second week post-calving (OR=2.9, P=0.05).[16] Thus, measuring and monitoring the dry matter intake in the close-up group every week has

Table 1. 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%	23%	81%	83%	35%	91%	68%	48%
Ketocheck‘ (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.

utility. However, beware of group demographics relative to time of expected calving and parity, which can influence these parameters dramatically. Fresh cow intakes are generally less useful because we are primarily interested in the intakes of cows within the first three weeks post-calving. If a fresh cow group exists, it is often composed of cows that may be several months post-calving. Larger farms are more likely to have more useful opportunities for measuring dry matter intake because of the ability to group cows into parity and smaller days-in-milk windows.

DHI test day data

Since milk fat and milk protein percentages are altered in sublinical ketosis, these parameters have been investigated for their utility in defining subclinical ketosis. Among all protein and fat parameters, a protein-to-fat ratio of ≤ 0.75 was the best test for diagnosing subclinical ketosis at the cow level in a Canadian study.[4] However, the protein-to-fat ratio was not a good test overall, having a sensitivity of 58% and a specificity of 69%. There is good European data that supports using milk acetone measured in routine Dairy Herd Improvement (DHI) samples. A big problem with both this and protein-to-fat ratio is the frequency of sampling. Subclinical ketosis is prevalent in the first few weeks postpartum. However, DHI testing frequency is typically every 30 to 40 days. Thus, the interval of sampling is too infrequent to hold great utility. However, incorporation of milk acetone into in-line sampling methodology that could be done daily holds tremendous promise.

Identifying high risk herds

Herd incidence of certain diseases may be useful to decide whether a herd has a problem with subclinical ketosis. Using data from a 25-herd study conducted in Guelph in 1995/1996, the median cumulative herd incidence of subclinical ketosis was 41% in the first two months post-calving, which crudely broke down into a threshold of 20% in week 1 and week 2 post-calving. Summary data for each herd from each cow's first DHI test post-calving was used to assess the protein-to-fat ratio as a herd-level test for classifying a herd as a high- or low-incidence herd for subclinical ketosis. If more than 40% of cows in the herd at first DHI test had a protein-to-fat ratio of less than or equal to 0.75; those herds were likely to be problem herds. This test had a sensitivity of 69% and a specificity of 83%. Although more work needs to be done on herd-level indicators of subclinical ketosis, herd-level protein-to-fat ratios appear to be better indicators of herd level issues than individual cow protein-to-fat ratios are of identifying cows with subclinical ketosis problems.

Additional analysis indicates that herd incidence of displaced abomasum is positively associated with the probability of a herd having a high incidence (>20% in the first two weeks of lactation) of subclinical ketosis. In addition, if herds had greater than 10% of transition cows with a BCS ≥ 4.0 at three weeks pre-calving, that herd was extremely likely to have a problem with subclinical ketosis.

Economics of Monitoring

For the herd-level monitoring interpretation, the savings achieved lie in identifying a problem sooner rather than later, since nearly all problems will eventually be identified. A conservative estimate of the economics of a biweekly program suggests that a routine monitoring program would pay back if one major problem was identified earlier than traditional means every four to five years. The economics of individual cow testing depend on efficacy of treatment, accuracy of the test, cost of therapy and prevalence of disease.[7]

Conclusions

Given the cost of subclinical ketosis, the fact it is a common problem in early lactation, and the strong association with clinical disease, monitoring programs for subclinical ketosis during the first few weeks of lactation may be warranted. There are several cowside tests for subclinical ketosis available, however, all of the current tests have both strengths and weaknesses. The appropriate design and frequency of a subclinical ketosis monitoring program will depend on the purpose of the program and the frequency of disease within the herd. Utilization of a technician in peripartum monitoring programs might be a way to ensure compliance and benefit both the herd and the veterinarian.

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