Metabolic Profiles for Evaluation of the Transition Period

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Abstract

Metabolic profiling as originally defined using the Compton Metabolic Profile procedure was insufficient for identifying herd problems. More recent research has better characterized metabolic dynamics around the time of calving and their relationship with high prevalence of metabolic and infectious disease problems. This, coupled with a series of factors including improved diagnostic tests, increasing herd size and recognition of transition cow health impact on herd performance, have revived interest in new testing protocols to assess herd periparturient disease risk. Cost and interpretation have been the limiting factors to widespread adoption of a metabolic profiling procedure. New strategies to reduce costs and yet maintain some useful diagnostic value have evolved either herd-based diagnostic or screening approaches. Use of pooled samples collected from pre-defined physiologic groups, and testing a wide array of analytes, have been advocated as a practical and economical method of screening the dairy herd for periparturient disease risk. Key diagnostic analytes identified as predictive for transition cow problems include non-esterified fatty acids (NEFA), NEFA:cholesterol ratio; β-hydroxybutyrate, albumin, calcium, sodium and magnesium. The current challenge is development of diagnostically valid interpretation criteria for pooled samples. Most importantly, it must be remembered that metabolic profiles are almost useless without being coupled with animal and facility evaluations, body condition scoring and ration evaluation. The combination, used within a team approach, can be an extremely useful diagnostic tool in nutritional evaluations of the dairy herd.

Résumé

L'établissement'de profils métaboliques, tel que défini à l'origine avec la méthodologie de Compton, est insuffisant pour l'identification des problèmes dans un troupeau. Des travaux récents ont permis de mieux caractériser la dynamique péri-partum des métabolites et leur association avec une prévalence élevée de maladies métaboliques et infectieuses. Cette connaissance jumelée à d'autres facteurs, incluant de meilleurs tests diagnostics, une plus grande taille des troupeaux et la reconnaissance de l'impact de la santé de la vache en période de transition sur la performance du troupeau, a ravivé l'intérêt à développer de nouveaux protocoles pour déterminer plus adéquatement le risque de maladie péripartum dans un troupeau. Les coûts et l'interprétation des profils ont limité l'adoption à grande échelle de la procédure des profils métaboliques. De nouvelles stratégies qui réduisent les coûts tout en maintenant une certaine valeur diagnostique ont été développées avec une approche de diagnostic de troupeau ou de dépistage. L'utilisation d'échantillons composites recueillis à partir de groupes physiologiques prédéfinis de même que le test d'une grande panoplie de métabolites sont mis de l'avant afin de développer une méthode pratique et économique de dépistage des maladies péri-partum dans un troupeau laitier. On retrouve les acides gras non-estérifiés, le rapport des acides gras non-estérifiés au cholestérol, le bêta-hydroxybutyrate, l'albumine, le calcium, le sodium et le magnésium parmi les métabolites diagnostics clef prédisant les problèmes chez les vaches de transition. Le défi maintenant est de développer des critères d'interprétation pour les échantillons composites qui sont valides au point de vue diagnostique. De façon plus importante, on doit se rappeler que l'établissement de profils métaboliques est presque sans intérêt sans évaluation conjointe de l'animal, du logement, de la ration et l'évaluation de la condition corporelle. Cette combinaison, utilisée dans le cadre d'une approche d'équipe, peut être un outil diagnostic extrêmement utile dans l'évaluation de la nutrition dans un troupeau laitier.

Introduction

Blood tests from individual animals are routinely used to diagnose disease problems in dairy cattle. Veterinarians, producers and nutrition consultants alike seem to be interested in extracting pertinent information relative to herd nutrition and health status from blood tests. The Compton Metabolic Profile (CMP) has traditionally been used in this approach.[29] The original intent of the CMP was to 1) monitor metabolic health of the herd, 2) help diagnose metabolic problems and production diseases and 3) identify metabolically superior cows.[29,31]

The CMP involved collecting seven to ten blood samples from three predefined groups of dairy animals, i.e., dry, peak lactation and mid-lactation, and having selected metabolites measured.[29] From the test results, averages for each metabolite were calculated for each respective group and compared to reference values. Seven animals are considered the minimum number sampled for statistically significant interpretation. As one might expect completing 13 biochemical tests on 21 individual samples was extremely expensive ($200 to >$400 US), even with automated equipment. The CMP generally received positive endorsements as a diagnostic aid from studies outside the United States.[5,13,29] In contrast, results of metabolic profiles in studies completed in the US have generally been less than enthusiastic about their potential diagnostic value.[1,22,24] Application of this diagnostic procedure on a herd basis has been questioned relative to its validity and sensitivity in defining a problem, as well as its total cost. Unfortunately in many herd situations, blood analyses are used preferentially in lieu of other more appropriate diagnostic procedures such as ration evaluation and physical exams and without regard for proper technique to ensure sound diagnostic information. However, blood metabolite analysis can reveal some useful information if properly interpreted in conjunction with animal and ration evaluations.

Research since the time of CMP development has clarified many metabolic issues of the transition cow and its relationship to periparturient disease.[2,8,16] Additionally, the shift to increased herd size and recognition of the adverse consequences of periparturient disease has led to increased interest in a revised metabolic profile application in monitoring transition cow health and disease risk.[19] The objective of this presentation will be to review the application and interpretation of a modified metabolic profile procedure using pooled samples for use as a herd screening tool for assessing periparturient disease risk.

Metabolic Profile Rationale

A "metabolic profile" is defined as a series of specific analytical tests run in combination and used as a diagnostic aid.[21] Although similar samples and analytical methods are used in assessing disease diagnosis or metabolic profiling, their approaches to sampling and interpretation are different.[19,35] With disease diagnosis, one selects a small population of representative clinically affected animals for blood analysis. Results are compared to laboratory 95% reference ranges for interpretation. Disease diagnosis is based on a recognized pattern of changes in one or more blood analytes. In contrast for metabolic profiling, one collects samples from clinically "normal" individuals within certain defined physiologic groupings. Ultimately metabolic profiling is being used to evaluate disease risk in contrast to disease diagnosis, though in many situations one often slips into a disease diagnosis framework. Methods used in sample collection and interpretation will vary by the metabolic profiling procedure used. In contrast to methods of the past, metabolic profiling should be considered just one tool in the nutritional diagnostic toolbox. In the broadest sense of the terminology, a metabolic profile should also include evaluation of herd records, animals, facilities and rations and integrate the information into a final diagnostic assessment.[19,34] Results of a metabolic profile should be used to direct or focus attention of a diagnostic process (screening tool) or to help confirm presence of a herd disease process (diagnostic tool).

Diagnostic Profiling – Individual Sampling

In the traditional approach to metabolic profiling, a representative sampling of individuals were collected and analyses performed. With the CMP, a mean value for individuals was determined and then compared to some defined reference value. The problem with this approach was the amount of incurred costs and lack of test result sensitivity (interpretation problems). Oetzel has advocated that evaluating a proportion of individual samples relative to a given reference criteria may be of greater value in assessing disease risk.[28] Analytes with a threshold value above or below which is associated with disease risk are best evaluated as a proportion rather than a mean. For example, one could determine rumen pH to diagnose subacute ruminal acidosis in a herd. Elevated pre-fresh nonesterified fatty acids (NEFA) concentration (≥ 0.4 mEq/L) and postfresh β-hydroxybutyrate (BHB) concentration (≥ 14.4 mg/dl) are recognized risk factors for ketosis and left-displaced abomasum.[9,11,12,23,28] Low blood calcium concentration (< 8 mg/dl) immediately post-calving is a risk indicator of subclinical hypocalcemia.[28] Blood urea nitrogen (BUN) and urine pH have also been advocated as potential indicators for assessing herd protein status and anionic salt responsiveness, respectively.[27,28] These two values can be evaluated by using means of individuals.

Few would argue the strength of individual analysis in metabolic profile analysis. Indeed the gold standard for analytical analysis would be to measure a large percent of the population of interest as individuals. Statisticians suggest at least eight subsamples from a population is representative (mean analysis), though 12 samples are best for threshold analysis.[28,29] At the same time, statisticians also state if we really wish to better characterize the population, then more samples are better. Similarly, we can reduce required sample numbers by reducing the level of confidence needed in our results. As Oetzel describes, clinical decision making does

not require 95% confidence in a conclusion as does research, but 75% confidence in a result may be reasonable.[28] In some ways, this approach is a hybrid between using blood metabolite analysis for specific disease diagnosis and metabolic profiling. This is seemingly in contrast to the underlying premise of metabolic profiling, but may better reflect known disease relationships.

A second approach with individual sampling is more similar to the original metabolic profile test. To address concerns with metabolic profile testing costs, the number of determined analytes per sample is reduced. For example, the metabolic profile test offered by Michigan State University includes urea nitrogen, albumin, aspartate aminotransferase (AST), NEFA and BHB (Herdt TH, personal communication). Discount pricing is offered for submission of multiple tests (seven or more) to help reduce costs. Analytical testing addresses key metabolites reflecting energy balance, liver function and protein status and having documented association with disease risk. Samples can be collected from immediately pre-fresh and post-fresh animal groups, though few will take samples from more than one group (pre-fresh or fresh) due to costs. Again, test interpretation is on an individual basis relative to defined reference values and proportional risk assessment. This testing procedure provides more diagnostic screening capabilities, compared to a single-analyte diagnostic procedure. Thus, this approach may help provide direction to the diagnostic process. However, is there another way to assess herd metabolic status and disease risk and find a balance between economics and valid diagnostics?

Herd Screening – Pooled Samples

A pooled-sample approach was an attempt to address both cost concerns and increased scope of testing in assessment of herd nutritional or disease-risk status.[35] In the individual approach described above, single variables are being used to assess specific disease risks. A pooled-sample approach should be considered more of a herd screening process and not a specific diagnostic test. The approach to this application is to evaluate various aspects of integrated metabolism as well as changes that occur over the transition period. Testing is broad-based and includes analytes reflecting energy balance (NEFA, BHB), protein status (BUN, albumin), liver function (various enzymes, cholesterol, and triglycerides) and macromineral homeostasis (see discussion below).[35] This can also be expanded to include microminerals and vitamins. To measure this range of analytes, one must reduce costs. One approach is to measure analytes in pooled samples and evaluate over a series of predefined physiologic states (Table 1).

In the original protocol for the CMP, mean analyte values within physiologic groupings were used for interpretation. These mean values were arithmetically determined from individual samples. Use of individual sampling resulted in the high associated costs of this procedure. Can pooled samples be used to reduce the cost and provide some valid method of herd assessment? There are essentially two issues to be resolved in answering this question. First, can pooled samples accurately reflect the arithmetic mean of the individuals? Second, can one adequately define valid interpretation criteria for pooled sample values? The first question is the easiest to answer.

In a number of preliminary studies, individual samples were used to determine if pooled sample results for any given analyte were different from the arithmetic mean of the individuals within the pool.[33,37] Using

Table 1. Suggested physiologic groupings for collecting blood samples in completing metabolic profile testing using individual or pooled samples.

Physiologic Groups	Time relative to Calving	Parity	Disease Status
Far Off Dry	>10 days following dry off and < 30 prior to calving	Keep heifers and 2+ lactation animals separate – pool as separate parity groups within physiologic groups	Unknown
Close-up Dry	Between 3 and 21 days prior to calving (3 to 14 days best)		Unknown
Fresh	3 to 30 days in milk (7 to 21 days best)		Group cows with and without disease within lactational groups – keep days in milk similar within and between groups
Lactation groups	Define as needed based on disease conditions, production level or other problem		

a variety of statistical methods, we found that pooled samples do accurately represent individual means from pool sizes ranging from 5 to 20. When one uses sample sizes of 25 to 30, we found more statistical differences between arithmetic mean and pooled values, but numerical values were not greatly different. This statistical difference may have been more of a "statistical artifact" due to the larger "n" and smaller error term. In plotting arithmetic mean versus pooled value for various analytes, most of the graphs showed a nearly 1-to-1 relationship. These results are consistent with another study looking at a pooled sampling technique.[25]

Does this mean pooled values precisely represent the mean of individuals? No, there is some variation, though it depends upon the analyte. Fortunately, key metabolic indicators for transition cows showed less than a 5% difference between arithmetic mean and pooled values.[37] In our experience, pooled sample values are always in the same direction relative to the reference population mean, but may vary in magnitude in distance away from the mean. Samples that were collected and handled under poor or less than ideal conditions (various degrees of hemolysis present) resulted in increasing differences between pooled and mean values. We have also found that pooled samples held at room temperature for a period of time (hours) resulted in analytical problems resulting from sample gelling. Herds that were experiencing more disease problems had more statistical differences between pooled and arithmetic mean values.[37] From the preliminary studies, it would seem feasible that analyte analysis of a pooled sample does reasonably represent the arithmetic mean of the sampled individuals.

Pooled Profile Procedure

Proper sample selection and pooling technique are keys to success with pooled samples. In selecting samples, we need to ensure that true herd or nutritional differences will be borne out and not masked by random or other sources of controllable variation.[17] Samples can be pooled by appropriate physiologic states to allow interpretation of dynamic changes in "population" means over a period of time (Table 1).[35] For example, to address a fresh cow problem, pooled samples can be collected from recently dry cows (>10 days following dry-off up to 30 prior to calving), close-up dry cows (three to 21 days prior to calving) and fresh cows (three to 21 days-in-milk). Other appropriate sample pools can be determined given the specific problem to be addressed. For example, if a herd was experiencing increased prevalence of retained fetal membranes (RFM), you could pool both clinical and non-clinical animals within the fresh cow group for comparison. Obviously you would not know which animals go on to have RFM prior to calving. Also within these groupings, first lactation animals are kept separate from second and greater lactation animals. This physiologic grouping strategy is based on findings of significant differences in mean analyte concentrations across these groups in either healthy or sick cows.[36] Most clinical pathology laboratories do not alter their reference ranges relative to physiologic criteria.

Sample collection and handling are important if one is to expect useful diagnostic information in return. Blood samples should be taken from either jugular or coccygeal veins with a minimal amount of stress. Blood samples from the mammary veins are not appropriate, given the loss of nutrients into the mammary gland. Lower concentrations of phosphorus and potassium have been documented in jugular compared to coccygeal blood samples as a result of salivary gland uptake.[26,30] Time of sampling relative to feeding and feeding management may also influence metabolite concentrations and should be considered in the decision process of when to sample. If herds are being repeatedly sampled as a monitoring tool, samples should be taken at approximately the same time of day to minimize diurnal and prandial variation.

Vacuum tubes are the most common and easiest form of sample collection. A variety of vacuum tubes are available. Vacuum tubes are color-coded for specific diagnostic test procedures based on the specific anticoagulant or additive present in the tube. Most commonly, a single serum (red top, no anticoagulant) or plasma (green top, sodium heparin) sample is collected; however, in some cases a whole blood sample may be desired. Extreme efforts should be taken to prevent hemolysis of the sample. All samples should be properly identified with animal and group identification and date of collection. Other pertinent information for interpretation of the metabolic profile would include animal age, lactation number, milk production level, milk composition, days-in-milk, pregnancy status and body condition score.

By pooling samples you are obtaining information from a greater number of animals for much less cost. Rather than the standard 21 samples to calculate three group means, you may submit three pooled samples, which represent means of 10 to 20 animals each. For data from pooled samples to be relevant, all cows should be equally represented. In preparing pooled samples one must be meticulous in precisely measuring equal amounts of serum from each individual to be included in the pooled sample. Depending upon the total number to be included, typically between 100 and 500 µl (0.1 to 0.5 ml) from each individual are mixed into a new clean test tube (7 to 10 ml capacity). This process is best completed with use of a micropipetter or a TB syringe for precision. Pooled samples should be adequately mixed, then directly submitted to the laboratory or frozen and shipped.

Diagnostic Indicators of Disease Risk

Over the past 20 years, our understanding of transition-cow metabolism and its relationship to pathogenesis of periparturient diseases has expanded greatly. In concert with this improved understanding of integrated transition metabolism there has been an increase in technical methods to assess metabolic status. Together, these advancements have reinvigorated interest in metabolic profiling. Another driving force for renewed metabolic profiling has been increased awareness of the critical role periparturient disease plays in dairy farm profitability. Periparturient disease-associated culling and reproductive infertility, coupled with the obvious health and production concerns, are driving interest in predicting potential risk for disease. Most work in this area has focused on specific disease entities (i.e., milk fever, ketosis, left displaced abomasum); however, it is well documented that periparturient diseases are interconnected and not single entities.[7,14] Our preventive medicine focus should be directed to increasing the number of cows that complete the transition period without disease events of any degree. Currently, more than 50% of cows experience one or more periparturient diseases.[3,39] In most clinical situations there is tremendous need to assess risk of any disease versus no disease problems, as opposed to defining specific disease risks. The following discussion highlights key metabolic parameters that identify high risk of periparturient disease collectively.

Energy Balance

Energy balance is by and far one of the most critical nutritional factors impacting on animal health, lactation, and reproductive performance. Traditionally, we have monitored changes in energy balance via body weight and condition score changes over time. This procedure may not be a sensitive enough tool when dealing with the transition cow. However, body condition score monitoring is still an important management tool, especially in assessing body condition changes with lactational performance.

Nonesterified fatty acids (NEFA) have become the mainstay in determination of energy balance. Many research studies have shown good correlations between energy balance and serum NEFA concentrations. Serum NEFA concentration is the result of adipose tissue breakdown of fat in response to negative energy balance. Circulating NEFAs are absorbed and metabolized for energy by the liver and other tissues. Concentration of NEFA, then, directly reflects the amount of adipose (fat) tissue breakdown taking place. Excessively high NEFA concentrations due to negative energy balance results in fatty infiltration of the liver, which is associated with higher incidence of periparturient metabolic diseases.[6,10,20] The original reference values for NEFAs were based on data from Michigan State University Clinical Nutrition Laboratory suggesting NEFA concentrations above 0.4 (Close-up dry) and 0.6 (Fresh) mEq/L (mmol) are associated with four and five-fold increase of disease risk, respectively. These criteria have been supported by similar findings in other studies, and show increasing risk with higher concentrations.[23,39]

Another parameter useful in assessing energy status is ketone body concentrations. At present, measurement of β-hydroxybutyrate (BHB) concentration is most commonly used. However, BHB can come from dietary sources (poorly fermented silage) and not reflect aberrant metabolism. Concentrations of BHB < 26 mg/dl and > 14.5 mg/dl represent animals with subclinical ketosis. Those with concentrations ≥ 26 mg/dl are defined with clinical ketosis. Prior to calving, BHB concentrations generally do not exceed 6-8 mg/dl, unless the animal is in negative energy balance or consuming ketogenic silage. Following calving, BHB concentrations can become greatly elevated. Cows with BHB concentrations above 10 or 14 mg/dl are 3.2 and 4.3 times at greater risk for postpartum disease.[39]

Protein Evaluation

Assessing protein status is a bit more difficult than energy balance. At present, there is no single metabolite that can be measured which directly reflects protein status. As a result, multiple parameters are needed to assess protein status including BUN, creatinine, total protein, albumin and creatine kinase (Ck). Urea nitrogen concentrations are influenced by a wide variety of interrelated parameters, including dietary protein intake and rumen degradability, dietary amino acid composition, protein intake relative to requirement, liver and kidney function, muscle tissue breakdown and dietary carbohydrate amount and rumen degradability. Creatinine is used to assess renal function and its impact on BUN values. Total protein and albumin reflect availability of protein and their concentration decline in the face of protein deficiency. However, this occurs over a period of time. Albumin has a relatively short half-life and can reflect protein deficiency problems over a period of a month or two. Creatine kinase is released from muscle when it is catabolized or injured.

Albumin was found to be associated with postpartum disease and can be used to predict disease risk in close-up and fresh periods.[39] Fresh cows that could maintain serum albumin concentrations ≥ 3.5 g/dl were less likely to have postpartum disease. Serum albumin concentrations ≤ 3.25 g/dl in close-up dry cows resulted in a three-fold greater risk for postpartum disease. In spite of concerns about variables confounding albumin interpretation, it seems to be a good disease risk indicator, possibly reflecting availability of amino acids from the

labile protein pool. In most dairy herd dietary protein deficiency situations, BUN values will be low (<10 mg/dl) with normal albumin concentration (>3.5 g/dl) in the early dry cows. Close-up dry cows will have low to moderate BUN, lower albumin and elevated Ck values. Fresh cows generally have low BUN and low albumin (<3.0 g/dl). These fresh cows seemingly fail to properly respond to any disease insult. Protein deficient fresh cows will die from metritis, mastitis, foot rot, and anything else without antibiotic therapy.

Liver Function

We are all too familiar with the process of fatty infiltration of the liver in the transition cow.[4,15] Much has been written on the negative role of excessive fatty infiltration and incidence of periparturient disease. Fatty infiltration of the liver is a natural process for the dairy cow transitioning into lactation, but it must be under control. Liver function can be assessed through a variety of enzymes: gamma-glutamyltransferase [GGT], aspartate aminotransferase [AST], sorbitol dehydrogenase [SDH] and total bilirubin concentrations in the blood. Unfortunately, an elevation in any of these parameters does not mean anything more than some insult has occurred to the liver. Bilirubin values are more specific to bile flow problems than overt liver cell damage. These enzyme values need to be interpreted in conjunction with total cholesterol and NEFA results.

As described for energy balance, NEFAs are released into the circulation as a direct result of fat breakdown. The liver takes up NEFAs in direct relationship with their concentration in blood. Once in the liver, NEFAs can either be partially metabolized to ketone bodies, or distributed to other tissues for energy metabolism, or they can be used to synthesize fat. High NEFA values result in either elevated ketones or fat production by the liver. Fat in the liver has two potential options—remain in the liver cell and initiate hepatic lipidosis (fatty liver), or be transported out of the liver. In order for fat to be transported out of the liver, protein is required. Fat is transported in blood in compounds termed lipoproteins; this is the only way fat is soluble in blood. The lipoprotein structure that transports fat from the liver is identified as a very low density lipoprotein (VLDL). Associated with fat in the VLDL structure is a substantial amount of cholesterol. Therefore, total serum cholesterol indirectly measures the presence of VLDL in blood, and consequently measures the liver's ability to produce VLDL. If VLDL production is compromised, hepatic fatty infiltration will ensue. Therefore, low (<75 mg/dl, prefresh; 400 mg/dl, fresh) total cholesterol values characterize conditions in which VLDL production is limited and fatty infiltration is probable. Some investigators have suggested assessing the NEFA-to-cholesterol ratio for this reason.[20] Calculated NEFA-to-cholesterol ratio was predictive for postpartum disease in the close-up dry (>0.2) and fresh (>0.3) period pools.[39]

Macromineral Evaluation

Macrominerals calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), chloride (Cl) and sulfur (S) are of extreme interest relative to their role in milk fever, alert downer cows and weak cow syndrome. Unfortunately, most of these minerals are tightly regulated in the body through a variety of homeostatic processes. Blood concentrations of macrominerals are not reflective of dietary status when the homeostatic system is functioning properly.[18] Phosphorus, K, Mg and S are macrominerals in which blood concentrations are somewhat sensitive to dietary intake.[18] Sodium and chloride concentrations are altered when renal or digestive function is compromised or in extreme dietary deficiency states. Assessment of Ca concentrations around the time of calving is a useful indicator of how well the Ca regulatory system is working and potential for clinical or subclinical hypocalcemia problems.[28] It is perceived that other than the two weeks prior to and following calving, blood Ca is not a very useful diagnostic value as a result of the intact regulatory system. Therefore macromineral blood concentrations will need to be carefully interpreted in light of whether or not the homeostatic system is in proper operation.

In a recent retrospective study, a number of significant predictive relationships were found between serum mineral concentrations in the four weeks prior to or following calving and periparturient disease.[38,40] Most significant was the association between cows with pre- or postpartum calcium concentrations below 8 mg/dl. Either pre- or postpartum cows with serum total calcium < 8.0 mg/dl were four times more likely to have postpartum disease problems. Pre- and postpartum sodium concentrations were also highly associated with postpartum disease risk. These were interesting findings, and suggest macromineral concentrations may be more predictive than previously thought. Further work is needed to validate the relationships identified in this preliminary study.

Micromineral and Vitamin Evaluation

Assessment of trace mineral and fat-soluble vitamin status is routinely completed using direct blood concentration measurements. The question to ask is whether or not there is a predictive relationship between tissue and blood trace mineral or fat-soluble vitamin concentrations and presence of nutrient-specific deficiency disease. On the surface one would have to say yes, because we can document low nutrient concentrations in the presence of disease signs. The question really becomes one of how predictive mineral and vitamin

concentrations are, and which ones are the best indicators. To understand the issue here, we need to appreciate that trace minerals and fat-soluble vitamins are not in a single large pool in the body, but are distributed into a number of different pools, which have different functions and availability. The different nutrient pools described include a storage, transport and biochemical function pools.[32] As a result of the storage capacity for trace minerals and fat-soluble vitamins in the liver, moderate dietary deficiencies or short-term severe deficiencies can be overcome without any effect on the critical biochemical functions performed by the element in question. If the dietary insult is severe or prolonged enough to drain the storage pool, then some effects might be seen in the transport pool. Finally when the transport pool has been compromised, the biochemical function pool will be compromised, resulting in some dysfunction. It is only when the biochemical function pool reaches a critically low level that we see the overt clinical deficiency disease we learned about in textbooks. Before the clinical disease stage is reached, we will see problems associated with subclinical disease, including increased disease susceptibility as a result of compromised immune function. This is the bulk of the trace mineral and fat-soluble vitamin deficiency disease problems.

Interpretation of Pooled Samples

The real challenge for metabolic profiling is defining appropriate and sensitive reference values. This is even more of a concern in interpreting mean samples, especially pooled values. This has been the stumbling block for adoption of this procedure. One must recognize that this process, however well refined, will never be as sensitive as individual animal analysis. Interpretation criteria for pooled samples are still in the process of development and refinement, but basic concepts will be discussed.

For individual animals, metabolite values are compared to standard, laboratory-dependent reference values. These reference values generally represent a 95% confidence interval. This means that 95% of normal animals should have a given metabolite concentration within this range. This also suggests that 5% of the population will be outside of this reference range and still be normal, emphasizing the need to clinically evaluate the animal. A number of factors, most notably physiologic state and age, have been shown to influence blood metabolite concentrations. Most reference ranges do not account for these differences, and thus may confound direct interpretation. Having a thorough understanding of the physiologic regulation of a given nutrient is crucial to interpretation.

In contrast to individual animal samples, pooled mean metabolite values cannot be directly compared to reference ranges in the same way. When interpreting pooled samples, one needs to remember that a measured value represents a population with individuals above and below the mean. As a general rule, means of pooled samples should be near the midpoint of the reference range to be considered normal. For example, if serum total calcium (Ca) concentration for fresh cows is 9 mg/dl and the reference range is 9 to 12 mg/dl, this might be interpreted to suggest a potential problem with subclinical hypocalcemia, whereas it would be considered normal in an individual. The measured mean of 9 mg/dl represents a population with approximately 50% of the individual values above and below. This suggests that a number of individuals would have serum Ca concentrations below the normal range. Of course interpretation of metabolic profile results has to be considered in light of presenting problems in the herd. If the herd is experiencing clinical signs consistent with subclinical hypocalcemia, e.g., slow increase in feed intake and milk production, displaced abomasum and ketosis problems, this would be supportive evidence of the metabolic profile results.

Without population variance determinations in pooled samples, you cannot really determine how significant mean differences are. Yet, with many metabolites, like BUN, calcium, magnesium or glucose, you can eliminate the possibility that a single sample was sufficiently low or high to skew the mean. For low BUN values, it is difficult to have values approaching zero whereas for other metabolites, if the sampled cow had an extremely skewed value, it would have been exhibiting clinical signs and would not have been sampled. Metabolites with high variability (wide range of values; liver enzymes) will be of less diagnostic value as compared to low variability metabolites (minerals).

In following the original concept promoted in interpreting CMP samples, pooled samples can be compared to an expected healthy population mean (or median) and the associated population variance.[41] Population statistics were determined for various blood analytes in dairy cows having no evidence of periparturient disease at different defined periods relative to calving (far off dry, close-up dry and fresh).[36] The number of standard deviations away from the healthy population mean was determined for a number of pooled samples, using both arithmetic means and pooled values. The percent of abnormal values within a pool was determined using individual samples, compared to standard reference values for respective analytes. A linear relationship, though specific for each analyte, was found between percent abnormal values within a pool and number of standard deviations different from the mean resulting. In general, if a pooled sample value was less than 0.25 (range 0.1-0.5) standard deviations from the

healthy population mean, then < 10% of the individuals had abnormal values. Analyte-specific criteria can then be developed to estimate number of abnormal values with a pooled sample. This analysis is consistent with previous work on defining criteria for mean values and what many laboratories are using for metabolic profile evaluation criteria. Further work to establish specific pooled sample criteria is ongoing.

A second method to use and interpret pooled samples is by a modification of statistical process control. In larger herds, where sampling can be done more repetitively, one could collect samples on a monthly or bimonthly basis and plot the pooled sample results over time. Standard deviations from a healthy population, as described above, could be used to initially generate various sigma levels for evaluation. One can then graphically monitor metabolic status using one or more key analytes over time to assess potential risk for periparturient disease.

Summary and Conclusions

Traditional metabolic profiling of the dairy herd resulted in tremendous financial investment, with subsequent unsatisfactory results in many situations. A number of factors are responsible for individual and herd variation in blood metabolite concentrations, confounding interpretation. In addition, the cow has an exquisite system of checks and balances, which maintains normal physiologic function within a wide array of dietary and environment insults. As a result of these physiologic regulatory mechanisms, simple blood concentration analysis has not been highly rewarding in accurately assessing nutritional and fertility status. A new approach to metabolic profiling, which involves pooling larger sample numbers, specific animal selection relative to physiologic state and stage of lactation, has been examined in an effort to better interpret serum metabolite concentrations on a herd basis. Most importantly, it must be remembered that metabolic profiles are almost useless without being coupled with animal and facility evaluations, body condition scoring and ration evaluation. The combination, used within a team approach, can be an extremely useful diagnostic tool in nutritional evaluations of the dairy herd. Only when the whole picture is evaluated will the use of metabolic profiles produce useful diagnostic information.

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