

# **Metabolic Profiling: Assessing Nutritional Status of the Transition Cow**

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## **Take Home Messages**

- Metabolic profiles can be used to assess individual animal or herd nutritional status when used in conjunction with animal and ration evaluations.
- To assess a fresh cow problem, one should collect samples from far off dry, close-up dry, and fresh cows.
- Pooled blood samples can be used to minimize costs.
- Interpretation of pooled samples cannot be accomplished in the same method as with individual samples.

## **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 (10). 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 (10, 11). A “metabolic profile” is defined as a series of specific analytical tests run in combination and used as a diagnostic aid (7).

The CMP involved collecting 7-to-10 blood samples from 3 predefined groups of dairy animals, i.e., dry, peak lactation and midlactation, and having selected metabolites measured (10). 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 to be statistically significant for interpretation. As one might expect completing 13 biochemical tests on 21 individual samples is extremely expensive (\$200 to >\$400 US), even with automated equipment.

The CMP had generally received positive endorsements as a diagnostic aid from studies outside the United States (2, 5, 10). In contrast, results of metabolic profiles in studies completed in the US have generally been less than enthusiastic about their potential diagnostic value (1, 8, 9). 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. The objective of this presentation will be to review the application and interpretation of a modified metabolic profile procedure for use in the diagnosis of metabolic problems associated with the transition dairy cow.

### **Nutrient Profile Analysis Procedure**

The goal of any metabolite profiling is to obtain the "population" mean and determine dynamic changes over transitions. Larger sample size generally better represents the population. Initially, cost is the main deterrent to large animal numbers; however, why not pool samples since we are interested in the mean value and not individuals? Samples can be pooled by appropriate physiologic states to allow interpretation of dynamic changes in "population" means over a period of time. For example to address a fresh cow problem, pooled samples can be collected from recently dry cows (>7 days following dry-off), close-up dry cows (<2-3 weeks prior to calving), and fresh cows (< 45 DIM). Other appropriate sample pools can be determined given the specific problem to be addressed. 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 3 group means, you may submit 3 pooled samples, which represent means of 10 to 20 animals each. The only negative part to this variation is the loss of statistical evaluation, i.e., population variance. However, this is not a major limitation if properly interpreted. Recent research has shown that pooled samples have the same value for most metabolites compared to means of individuals (13). Proper identification of appropriate animal groups or pools is absolutely critical if one is to obtain useful data.

For data from pooled samples to be relevant, all cows should be equally represented. Samples should be drawn only from visually normal animals to more accurately represent the population for a nutritional status evaluation. If needed, you could pool both clinical and nonclinical animals within a given group for comparison. To be able to appropriately interpret changes from one physiologic state to another at a single point in time, all animals should have been exposed to the same diets and management environments. This means to say that the fresh cows sampled today received the "same" diet that the early dry cows are currently receiving. If a dietary change was made recently, then comparisons between physiologic states may not be appropriate. If no changes were made, then compare dynamic changes in the "population" means for specific metabolites in accordance with clinical signs and ration evaluation.

### **Energy Balance Assessment**

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. Another parameter that might be useful in assessing energy status is ketone body concentrations. At present measurement of beta-hydroxybutyrate (BOHB)

concentration is most commonly used. However BOHB concentrations may not be sensitive enough and can come from dietary sources. A third method is a traditional research procedure, which has recently received much interest in the field. This is measurement of nonesterified fatty acids (NEFA) as a 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 (3, 4, 6). Reference values for NEFAs are based on data from Michigan State University Clinical Nutrition Laboratory (Table 1). Clinical experience suggests serum NEFA concentrations to be more sensitive to energy balance changes compared to body condition scoring in transition cow situations.

**Table 1.** Suggested serum values for total cholesterol and nonesterified fatty acids (NEFA) in the periparturient dairy cow.

Serum Metabolite	Early Dry	Close-up Dry	Fresh Cow
Total Cholesterol, mg/dl	> 80	> 75	> 100
NEFA, mEq/L	< 0.325	< 0.40	≤ 0.6

### 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 blood urea nitrogen (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. In most 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 (<2.5 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. An interpretation of this situation is that there are no amino acids

available to support the immune system and it fails, predisposing the animal to any bug that comes along.

### **Liver Function Evaluation**

We are all too familiar with the process of fatty infiltration of the liver in the transition cow. 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] and 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 most 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 NEFA 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 they are 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 the values described above represent total cholesterol values that 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 (6).

### **Macromineral Evaluation**

Macrominerals calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), chloride (Cl), and sulfur (S) are of extreme interest as to their status 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. Phosphorus, K, Mg, and S are macrominerals in which blood concentrations are somewhat sensitive to dietary intake. 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. Other than the 2 weeks prior to and following calving, blood Ca is not a very 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 lieu of directly measuring macromineral blood concentration, measurements of parameters directly related to functioning and responsiveness of the homeostatic regulatory system may offer some insight as to nutritional status. Other methods of determining mineral balance such as urinary excretion patterns are being investigated as more sensitive indicators of nutritional status.

### **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 (12). 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 we reach the clinical disease stage, 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.

The next issue to address is the ability of the chosen marker to be measured and its relationship to changes in one or more of these trace mineral or fat-soluble vitamin pools. Most of our markers currently being used are element concentrations in either whole blood or serum. These probably reflect the transport pool and not necessarily the biochemical function pool. As a result they may not be highly correlated with the presence of clinical disease. A good example here is serum copper concentrations. Unless serum copper is a critically low value, it has no significant predictive value in assessing potential for copper deficiency disease. Another example is the debate between serum and whole blood selenium values. Serum selenium values represent the transport pool and are very sensitive to dietary changes and liver mobilization. On the other hand, whole blood selenium values represent both transport and a portion of the biochemical function pools. This measure is somewhat less sensitive to dietary changes as a result of the greater proportion of whole blood selenium being present as the erythrocyte enzyme glutathione peroxidase. If one were to assess a potential response to a dietary change, serum selenium values would respond within a day or two while whole blood

may take a month or more to show a significant change. This could dramatically impact on your interpretation of the dietary response.

Liver mineral concentrations are good markers for the storage pool; however, they are not always highly associated with the presence of disease. Liver mineral concentrations may give us some insight into the adequacy of the mineral program and potential for disease. One additional avenue here is the assessment of mineral status in fetal and neonatal animals. Research has shown that the fetus can concentrate trace minerals in its liver and therefore, comparison to adult values is inappropriate. Secondly, fetal liver has a lower dry matter content than the maternal liver further substantiating the inability for direct comparison. Databases determining normal trace mineral concentrations in the fetal and neonatal liver need to be developed. Obviously we are a long way away from accurately predicting the potential presence of trace mineral deficiency disease problems with our current methodologies. A number of more predictive markers for specific nutrient pools need to be identified.

### **Metabolic Profiles: Interpretation of Results**

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. For trace minerals, blood or serum concentrations are buffered from acute changes as a result of dietary problems through mobilization of storage mineral, usually from the liver. This suggests that liver trace mineral status may be a better indicator of dietary adequacy, whereas measurement of a mineral-specific enzyme activity better reflects the presence of overt clinical deficiency disease compared to blood concentrations. Many trace mineral concentrations in blood are influenced by disease. As we come to better understand the factors that affect metabolites, we can adjust and better assess nutritional status.

In contrast to individual animal samples, pooled mean metabolite values can not be directly compared to reference ranges in the same way. When interpreting pooled samples one needs to remember that 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, you cannot really determine how significant mean differences are. Yet, with many metabolites, like BUN, Ca, Mg 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) will be of less diagnostic value as compared to low variability metabolites (Table 2).

**Table 2.** Categorization of blood metabolites relative to their range of values (variability) and diagnostic value.

<b>Low Variability High Diagnostic Value</b>	<b>Moderate Variability and Diagnostic Value</b>	<b>High Variability Low Diagnostic Value</b>
Albumin, Total protein	Cholesterol	Creatine kinase
Calcium, Phosphorus, Magnesium	BUN	Liver enzymes
Sodium, Chloride, Potassium	Glucose	
NEFA	Ketones	

## Summary

Traditional metabolic profiling of the dairy herd resulted in tremendous financial investment with subsequent unsatisfactory results in many situations. A variety 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. It is only when the whole picture is evaluated will the uses of metabolic profiles produce useful diagnostic information.

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