DO METABOLIC PROFILE TESTS IN DAIRY HERDS PROVIDE ANSWERS, OR JUST MORE QUESTIONS?

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Aim of presentation

The aim of this presentation is to discuss in general the use of blood analysis for nutriture assessment. The utility, challenges, sampling strategies, and methods of interpretation will be discussed collectively with only a brief mention of specific analytes as examples. A table including some information about a few specific analytes is included at the end of this paper.

Concept of "nutriture assessment"

Nutriture is defined as "the status of the body in relation to nutrition, generally or in regard to a specific nutrient..." (Dorland's Illustrated Medical Dictionary, 24th ed.) I don't think this is a foreign concept to anyone in animal production. Important variables in nutriture assessment include growth rate, body condition, back-fat thickness, milk production, and many others. **These are all measures of nutriture assessment.** Measurement of blood component (analyte) concentrations is an additional means of nutriture assessment. Again, I don't think this is a foreign concept. Literally thousands of research papers could be cited in which nutritional treatments have been evaluated, at least in part, by responses in blood composition. The challenge is in application of blood analysis for nutriture assessment under commercial production situations. The major considerations are in the selection of analytes to measure, and in the interpretation of results. There are both biological and statistical considerations in the interpretation of blood analyte concentrations for nutriture assessment.

Biological factors in the nutritional interpretation of blood composition

An understanding of the physiology and metabolism of the blood analytes measured is essential for appropriate nutritional interpretation. Interpretation is often not straightforward. One critical biological factor with a large influence on interpretation is the presence or absence of disease.

The difference between disease diagnosis and nutriture assessment

There is an important distinction between the use of blood analysis for disease diagnosis and for nutriture assessment. First, disease diagnosis is intended to measure pathological (abnormal) variation, which is usually much greater than physiological (normal) variation. It is generally physiological variation that is of interest in nutriture assessment. Much more important, however, is the effect that disease, be it infectious, metabolic, toxic or other, can have on a wide variety of blood component concentrations.

Figure 1 represents the results of a blood chemistry analysis done on a down fresh cow. The scale is arbitrary, but the light gray boxes represent the "normal range," as defined by the clinical pathology laboratory. The darker lines represent the values measured in this animal. Note in this example, all of the values are outside of the defined normal ranges. In looking at these results, we might suppose there are important nutritional problems with this animal, and with the herd from which she came. The NEFA concentration is too high, indicating negative energy balance. The zinc concentration is too low, suggesting a dietary zinc deficiency. We could go on to suppose from these results that there are numerous nutritional problems. In fact, it is quite likely the cow has milk fever and her primary problem is hypocalcemia. All of the other abnormalities in this figure could be explained as secondary effects to hypocalcemia. If, based on these results we added zinc oxide to the ration of this herd, we probably made a mistake!

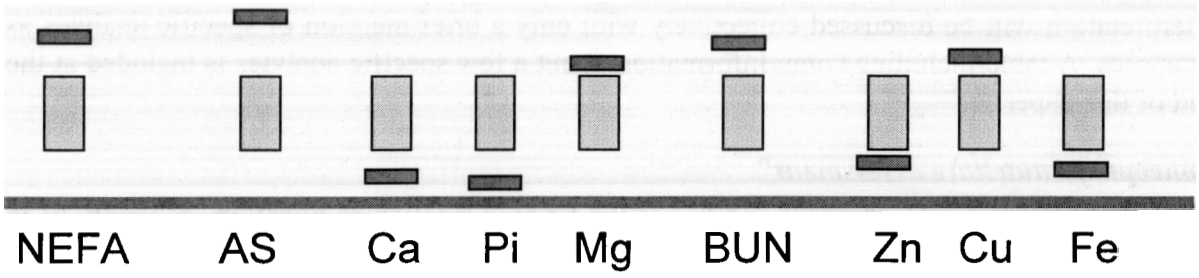


Figure 1. Blood chemistry analysis from a single cow, down after freshening.

Contrast the previous situation with Figure 2. In this case, light gray boxes still represent a reference or "normal range," but the dark lines represent the average values from seven apparently healthy cows. Note that all of the values are in the reference range, except for urea nitrogen (BUN). Elevated BUN can be a sign of severe dehydration or kidney disease, however it is quite unlikely that seven apparently healthy cows are all severely dehydrated or have kidney disease. It is much more likely that the high BUN represents a physiological variation and the values are in this range due to absorption of large amounts of ammonia from the rumen. It is quite possible that this herd would benefit from an adjustment in the ratio of rumen available-energy to rumen available-nitrogen.

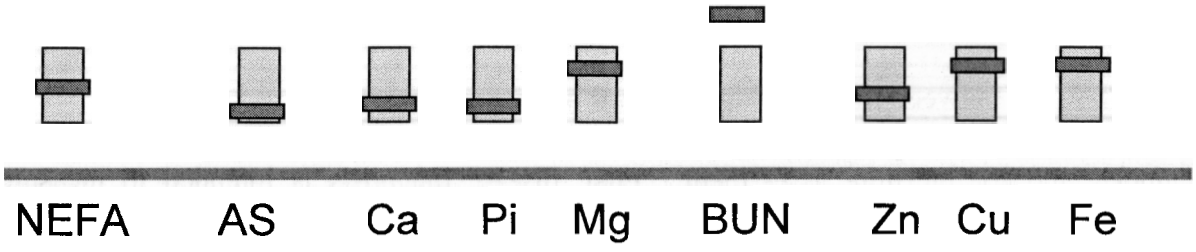


Figure 2. Average values of serum chemistry analyses from seven apparently healthy cows in a single herd.

The major point of this example is that samples taken from diseased animals cannot be used to make general nutritional inferences. Even in those cases in which animals have what appears to be a nutritional disease, samples should be taken from other apparently healthy animals to verify that the problem extends beyond a single individual, and is therefore likely to be related to diet.

Factors affecting physiological variation are often complex

In some cases, such as that for selenium, the concentration in the blood or blood serum is a reasonably direct indicator of dietary intake. For many other metabolites and minerals, the relationship between nutrition and blood concentration is more complex. The serum concentration of iron is a good example. Dietary iron intake can affect serum iron concentration, but so can other factors. One is the protein status of the animal. Serum iron is carried on a short-half life protein known as *transferrin.* The serum iron concentration is affected by the availability of transferrin, which is in turn related to protein status of the animal, among other things. Therefore, a low serum iron concentration in an apparently healthy animal might mean low iron status, but it might also mean a low protein status. Many similar examples exist, making it important to understand the basic metabolism of each blood analyte measured.

Statistical considerations in the nutritional interpretation of blood composition

Results of controlled research projects prove that diet and nutriture affect blood composition. However, *controlled research* implies that careful measures have been taken to minimize extraneous variation, thus maximizing the likelihood that a treatment effect will be detected, if one exists. Farm conditions don't resemble the conditions under which controlled research is conducted and the challenge in using blood component analysis for nutriture assessment under commercial conditions is in minimizing extraneous variation.

To understand this challenge, let's first consider the sources, or components, of variation in blood composition of animals. These could be listed as follows

- Random biological variation
- Genetic variation
- Circadian and/or prandial variation
- Seasonal variation
- Variation associated with physiological state (growth, gestation stage, lactation stage, etc.)
- Variation associated with pathological state (the effect of existing disease)
- Artifactual variation due to sampling or sample handling technique
- Analytical variation
- Environmental variation (influences external to the animal, including nutrition and other management factors)

Of this list, it is only the last item in which we are interested for nutriture assessment. All of the other sources of variation are extraneous to the purpose of nutriture assessment.

One of the ways in which extraneous variation can be limited is through sampling strategy. This involves primarily the selection of animals and the timing of sampling, but should also include the type of sample taken and the way in which it is handled.

Pathological variation, as mentioned above, is controlled by selecting only apparently healthy animals, and also by sampling several animals because it is unlikely that all will be suffering from a disease. Grouping animals by physiological state, such as age or lactation stage, controls physiological variation. Collecting blood samples at a fixed time relative to feeding controls for prandial variation, and careful attention to proper sample handling should eliminate variation due to sample handling errors.

Variation due to genetics, analytical technique, and unexplained biological randomness is not subject to control under farm conditions. However, sampling multiple animals can minimize the effects of these sources of variation on the interpretation of test results. The optimum number of animals to test is seldom known precisely, and indeed will vary with the user's definition of *optimum.* However, testing seven animals is a reasonable thumb rule, when more specific information about a given analyte is not available. Remember, this number is to be applied after the grouping has been decided upon. Three animals from one age group and four from another doesn't constitute a group of seven animals in this context; seven should be sampled from each group! If analytical cost is a major concern, the most critical group should be selected for sampling, rather than skimping on the number of animals per group.

Variability characteristics associated with specific analytes

There are variability characteristics associated with each specific blood analyte that give clues to its potential usefulness in nutriture assessment. One of these characteristics is the physiological range. The blood concentration of some analytes is under strict homeostatic regulation. In these cases, there is a relatively narrow physiological range and homeostasis limits the effect that environment (including nutrition) can have on the blood concentration. In figure 3 note that there appears to be much less rigid homeostatic control of blood NEFA, compared to blood glucose. This suggests that blood NEFA has greater potential for nutriture assessment than glucose. The breadth of the physiological range is related to the coefficient of variation for the analyte. Coefficients of variation for selected analytes is given in the tables at the end of this paper.

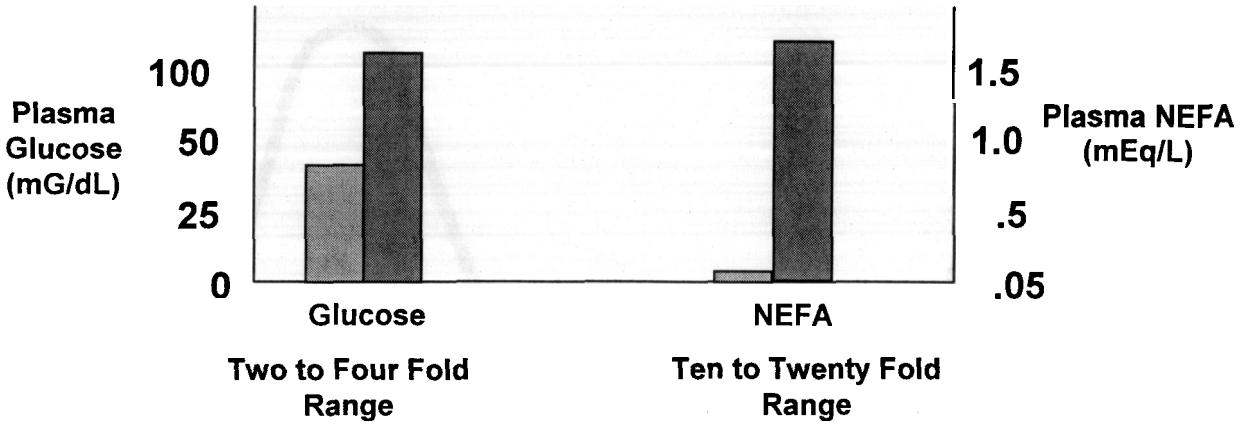
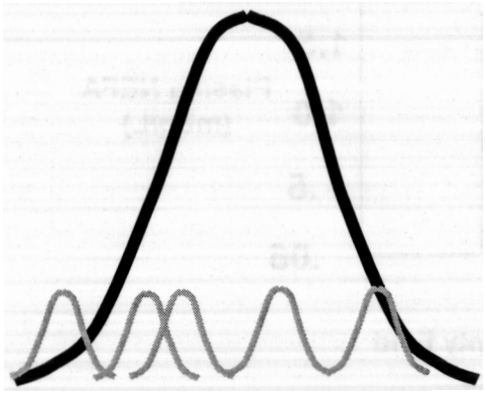
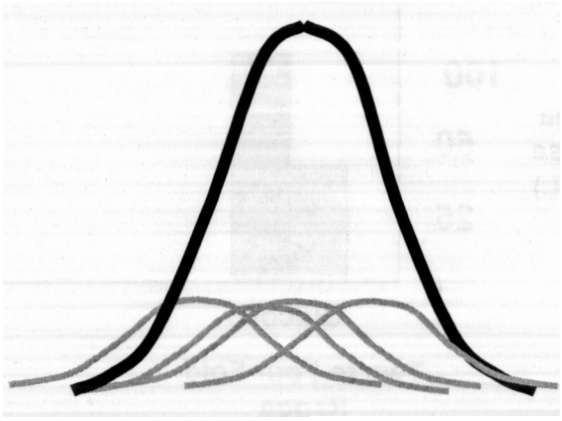


Figure 3. Comparison of the physiological range of glucose and NEFA (light bar = lower limit, dark bar = upper limit). Notice the much larger normal range for NEFAs compared to glucose. Thus there is relatively less homeostatic regulation of plasma NEFA concentration, compared to glucose. This allows environment (including nutrition) to play a larger role in blood NEFA concentration, compared to blood glucose.

Examination of variability components gives another hint to the potential usefulness of various analytes for nutriture assessment. Statistical techniques exist that divide variance into components. The concept of variability components is illustrated in Figure 4. With respect to nutriture assessment, an important variance component is the proportion of variability due to herd. When this proportion is high it means 1) there is a large effect of environment (probably nutritional) on the blood value in question and 2) there is a reasonable chance of detecting change with a relatively small number of samples. A list of variance components due to herd for some select analytes is given in the tables at the end of this paper.

Figure 4. An illustration of variance components. The large curves represent the population distribution for a variable, and the small curves the distribution within individual herds. In the figure on the right, there is a great deal of variability within herds, resulting in substantial overlap of the individual herd distributions. Variability within herd accounts for a major portion of the population variability, making variability due to herd a small portion of the total. In the figure on the left there is a similar population distribution, but here there is relatively little variation within herd and a large proportion of variability due to herd. Blood analyte concentrations with distribution characteristics like the figure on the left have much greater potential for nutriture assessment than do those distribution patterns like the figure on the right.

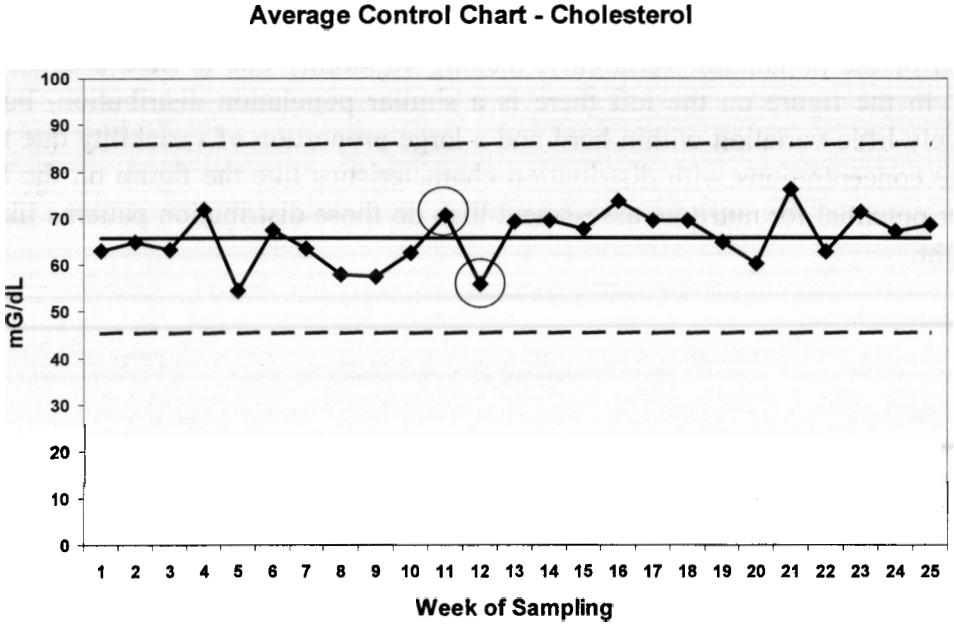




Interpretation

Interpretation of blood analyte concentrations for nutriture assessment requires that there be some frame of reference available, i.e. a scale by which to evaluate the results of a test. Numerous textbooks exist with reference ranges for clinical chemistry variables for cattle. These include such standard references texts as the Merck Veterinary Manual. The reference ranges given by such texts are intended to be used in disease diagnosis of individual animals and they are based on the need to exclude a very high portion of normal individuals. They are of little usefulness in nutritional assessment.

An alternative to the use of frequency distributions from individual animal values for the construction of reference ranges would be to use frequency distributions of herd means. A comprehensive listing of such ranges, to my knowledge does not exist, and if it did there would be constant concern about the appropriateness of the population of herds from which they were based. For example, if the herd in question has a 30,000-pound rolling-herd average, is it appropriate to compare it a reference range based on herds of lower milk production.



Perhaps a better means of establishing reference ranges is by considering correlative information comparing blood analyte concentrations to nutritionally related outcomes. One outcome of particular interest in dairy cows is the incidence of peripartum metabolic diseases. In this kind of a comparison, blood analyte concentrations are analyzed as "risk factors" for a disease. Risk factors can be negative or positive, indicating that higher blood concentrations of the analyte in question either decrease or increase the risk of disease, respectively.

Table 1 below is an example of a multiple logistic regression. These are the final variables in a model that was built from seven original variables. The data were from 1170 cows distributed among 67 dairy herds in Michigan (Cameron et al., 1998). Cows were classified as having elevated plasma NEFA concentrations if values were above 0.3 mEq/L. The analysis says that dry cows having plasma NEFA > 0.3 mEq/L are slightly more than twice as likely to develop a displaced abomasum after calving than dry cows with plasma NEFA less than 0.3 mEq/L. The exact value of 0.3 mEq/L is not as important as the observation that lower NEFA is good and higher NEFA is bad, with respect to the occurrence of displaced abomasum.

Table 1. Multivariable logistic model with random effects for individual cows for the occurrence or nonoccurrence of displaced abomasum.

Variable	Sign	P	Risk Ratio
Body Condidion Score	pos	0.001	2.405
Winter season	pos	0.002	2.967
Elevated NEFA	pos	0.007	2.042
Lactation number	neg	0.045	0.8075

This general approach needs to be applied to additional blood analytes and additional diseases, but is far more likely to yield meaningful interpretations than are other statistical methods.

Another approach to the evaluation of blood analysis for nutriture assessment is the use of *Statistical Process Control* (SPC) charts. Use of SPC has been applied recently to the evaluation of blood variables in dairy herds. This is a fairly simple and straightforward technique that may have application in larger herds. The utility of SPC is in its ability to track changes within a herd and to separate variance components. Figure 5 is an example of a *process control chart,* the basic tool of SPC. This example chart is of cholesterol concentrations in pre-fresh cows in a large Michigan dairy. There is not time in this presentation to discuss SPC in detail. Briefly, each point on the chart is the mean of seven animals sampled on the same day. In this case samples were taken weekly, and different animals sampled each week. The central line is the grand mean of all samples. The two dotted lines are referred to as *control limits.* When values extend past the control limits, it indicates the presence of an additional variance component, i.e. the change is significant. Variation within the control limits is considered to be random and to occur due to variance components that are constant within the herd.**Figure 5.**

Process control chart based on average serum cholesterol concentrations from pre-fresh cows in a large Michigan dairy. Each point represents the mean of seven animals sampled at the same time. The solid line is the grand mean of all the samples. The two dotted

lines are the upper and lower control limits. The upper and lower control limits are functions of the inherent variability in the system, and the number of animals tested per point. Weekly points falling outside of the control limits indicate that an additional variance component has been added, i.e. that something has changed and caused the variation. Variability among points within the control limits is considered random variation inherent to the system, i.e. one should not look for "causes" of variation within the control limits.

An important point to keep in mind about process control charts is that the control limits do not represent anything biological, i.e. it is not inherently good or bad that values are outside the limits. **It only means that something has changed.** Whether the change is favorable or unfavorable depends on a biological interpretation that requires some idea of what range of values is desirable. Process control charts appear to offer a potentially powerful way to monitor nutriture through blood sampling. They appear to be most useful in large herds where there are adequate numbers of animals for optimal groupings, there are sufficient animals over which to spread the testing cost, and the cost-benefit ratio is potentially very high.

Specific analytes

Metabolites and organic components

At the Diagnostic Center for Population and Animal Health (formerly the Animal Health Diagnostic Laboratory) at Michigan State University we offer a *metabolic profile* to assess certain aspects of the metabolic and nutritional status of *transition cows,* defined as cows between three weeks before and three weeks after calving. Analytes in this profile consist of plasma non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHB, a ketone body), albumin, urea nitrogen (SUN, or BUN), and aspartate aminotransferase (AST). These analytes were chosen because of their specific utility in the evaluation of cows in this period. A summary of their characteristics for nutriture assessment is in Table 2. Other organic analytes in blood may be useful in other situations.

Minerals

There is generally a large interest in assessment of mineral nutriture based on blood samples. Important considerations in the utility of blood mineral concentration in nutriture assessment are the relative degree of homeostatic control of serum concentrations, and the means by which homeostatic control is achieved. For some minerals homeostasis is regulated at the level of absorption. Examples are calcium, iron, and zinc. Absorption of these minerals is adjusted to meet body needs. When nutritional status is adequate, absorption of the mineral from the gut is nearly shut down. Thus, the mineral never makes it into the blood to affect serum concentrations. In the case of minerals such as these, blood concentrations are generally poor indicators of nutritional status.

This is in contrast to minerals like selenium and magnesium. These minerals absorbed in a relatively unregulated manner and homeostasis is achieved by renal excretion of the

excess mineral. In these cases, blood or serum concentrations are good indicators of nutriture because the excess mineral must travel through the blood on its way to renal excretion.

The Toxicology Section of the Diagnostic Center for Population and Animal health offers a battery of mineral analyses as a panel. The potential utility for nutriture assessment for serum concentrations of some minerals in the panel is listed in Table 3.

Summary

Analysis of blood samples has variable utility for assessment of nutriture, depending on the specific analyte in question. The major challenges in applying blood sampling as a nutriture assessment technique in the field are in minimizing extraneous variation. Important techniques for minimizing extraneous variation are selecting apparently healthy animals, grouping animals for sampling, sampling at fixed times with respect to feeding and milking, and sampling multiple animals (usually at least seven per group). Rigid reference ranges are not known, and may not be knowable. Associative statistical techniques, such as logistic regression, may be very useful in evaluating specific analytes for their utility in nutriture assessment, and in establishing reference values. Additional, on-farm statistical techniques are needed to aid in the interpretation of blood results. When picking out blood analytes for testing, it is important to consider metabolic and physiological factors influencing serum concentrations, the variability characteristics, the type of sample needed, and the sample handling necessary to achieve accurate results.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Beta hydroxy butyric acid (BHB, a ketone body)	Concentrations are normally elevated in early lactation, compared to other times. Concentrations increase excessively in the presence of large negative energy balance in combination with low availability of glucose precursors.	Select seven healthy appearing animals in the first 3 to 4 weeks of lactation	Serum is the blood sample of choice. Chill samples. Concentrations are stable in chilled or frozen serum. Avoid hemolysis. In cattle without subclinical ketosis, values are highest 2 to 4 hours post feeding.	Concentrations greater than 10 to 12 mg/dl are consistent with subclinical ketosis. In well nourished herds it should be possible to maintain a subclinical ketosis prevalence of less than 20%	Early lactation: 33% Mid lactation: 34% Dry cows: 40%	Early lactation: 25% Mid lactation: 40% Dry cows: 43%
Albumin	In healthy animals, serum concentration is related to labile protein stores.	Select seven healthy appearing animals in the first three to four weeks of lactation, or the last week of gestation.	Serum is the blood sample of choice. Chill samples. Concentrations are stable in chilled or frozen serum. Avoid hemolysis.	Concentrations normally decline near parturition, so reference values based on cattle at other times are too high. Values in the range of 3 g/dL or above are consistent with good protein stores; values less than 2.5 g/dL are consistent with inadequate protein stores		

Table 2. Analytes in the Michigan State Transition Cow Profile. Contact the laboratory at 517 353 9312 for information on submitting samples.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Aspartate aminotransferase	Sensitive, but not specific, indicator of fatty liver infiltration	Select seven healthy appearing cows in the last three weeks of gestation, or first three weeks of lactation	Serum is the sample of choice. Activity is relatively stable in serum. Chill quickly after collection and freeze before sending to laboratory	Desirable values are less than 100 IU/L. Values are somewhat laboratory specific		
Urea Nitrogen (BUN)	Serum concentration is correlated with rumen ammonia concentration. Values are indicative of the balance of rumen available carbohydrate and nitrogen.	Seven animals in the same feeding group.	Serum is the blood sample of choice in non-lactating animals. Chill samples. Concentrations are stable in chilled or frozen serum.	Between 13 and 16 mG/dL		

Non-esterified fatty acids (NEFA)	Indicator of negative energy balance	Seven cows in the last 3 weeks of gestation, or first 3 weeks of lactation. Collect samples just before the normal time at which fresh feed is offered.	Samples should be chilled immediately upon collection. Plasma is a convenient sample because cells can be separated easly from chilled samples. Values are stable in frozen plasma	For dry cows between three weeks and three days before calving, goal concentrations are less than 0.3 mEq/L. For fresh cows more than 3 days in milk, goal values are less than 0.7 mEq/L.	Late gestation: 87% Early lactation: 110%	Late gestation: 43% Early lactation: 54%

Table 2. Continued.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Blood Selenium	Good indicator of nutritional adequacy	Seven animals of similar age	Uncoagulated blood, EDTA is a sufficient anticoagulant	For individual adults: 120 to 250 nG/mL Target herd mean: 185 nG/mL (ppb)	14.5%	69%
Copper	Very low serum concentrations are indicative of nutritional inadequacy, however serum copper is not a sensitive index of copper nutriture	Thirteen apparently healthy animals of similar age (Herdt et al., 2000)	Serum is the sample of choice	For individual animals: 0.6 to 1.0 mG/L (ppm) Target herd mean: 0.8 mG/L (ppm)	17%	33%
Zinc	Very low serum concentrations are indicative of nutritional inadequacy, but serum zinc is a very insensitive indicator of zinc nutriture	Ten apparently healthy animals of similar age	Serum is the sample of choice. Avoid contact with the usual types of rubber tube stopper. Use BD "royal-blue" stopper tubes	For individual animals: 0.8 to 1.4 mG/L (ppm) Target herd mean: 1.1 mG/L (ppm)		

Table 3. Selected minerals available in the Michigan State *Serum soluble elements* profile. Contact the laboratory at 517 355 0281. Coefficients of variation and herd variance components are from Herdt et al, 2000.

Analyte	Nutritional Implication	Grouping Strategy	Sampling Considerations	Reference values	Coefficient of variation	Percent of variation due to herd
Calcium	High level of homeostatic control makes serum calcium a very insensitive index of calcium nutriture. Sampling cows within 12 hours of calving is a good indicator of homeostatic ability	Ten apparently healthy animals. Fresh cow samples can be accumulated over time.	Serum is the sample of choice. Total calcium is an adequate analysis. Little further information is obtained from ionized calcium	Individual animals: 8.5 to 11 mG/dL Target herd mean: 9.5 mG/dL For fresh cows (<12 hr) values should stay above 7.5 mG/dL	6.6%	35.5%
Magnesium	Good indicator of nutritional status and dietary intake	Ten apparently healthy	Serum is the sample of choice	Greater thar 2 mG/dL or 20 ppm	11.3%	43%

Table 3. Continued.

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