Serum protein

Serum contains many different proteins, but the two components of diagnostic significance relative to the chemistry profile are albumin and globulin. Albumin is synthesized in the liver and is the protein primarily responsible for the oncotic pressure of plasma. A large portion of the globulin fraction is made up of immunoglobulins which are synthesized by lymphoid tissue. The ratio of albumin to globulin (A:G ratio) is fairly constant in healthy cattle (reference range 0.84 - 0.94). Most chemistry profiles include measured albumin and total serum protein, while the value for globulin is typically derived by subtracting the former from the later. One must remember that if plasma is used instead of serum, fibrinogen will be included in the value for total protein, and hence, the derived globulin value; this may obfuscate the interpretation of the total protein and globulin. The discrepancy cannot be totally rectified by simply subtracting the value for fibrinogen, which is often determined by refractometry, from the value for plasma protein on the profile because of the difference in methods of analysis. Because most reference ranges are established for serum proteins, it is our opinion that serum is the sample of choice when evaluating the blood proteins. More specific and detailed evaluation of the globulin fraction can be achieved using electrophoresis, radial immunoassay and other methods which will not be discussed here.

Hyperproteinemias can result from an increase in albumin, globulin or both. The only cause of hyperalbuminemia is dehydration. In dehydration, both albumin and globulin rise, but whether they exceed the reference range is determined by the degree of dehydration and the original protein concentration in the serum. Hyperproteinemias without dehydration is almost always the result of hyperglobulinenia. Globulin increases substantially with age in dairy cows. The difference between two year-olds and five year-olds was about 1.5 g/dl, potentially a clinically relevant difference. Causes of hyperglobulinenia include chronic inflammatory diseases (traumatic reticuloperitonitis, liver abscess, chronic pneumonia) and hepatic disease. In chronic inflammatory disease, the A:G ratio usually decreases because of an increase in globulin which is often accompanied by a small decrease in albumin. In chronic hepatic disease, the decrease in albumin may be more substantial. Serum globulin may be one of the most overlooked values on the routine chemistry profile. Changes in the hemogram are often rather subtle and transient in inflammatory disease of cattle, compared to other species. Therefore, the evaluation of serum globulin is of great value in chronic inflammatory disease.

In mature cattle, hypoprothrombinemia is usually the result of hypoalbuminemia or panhypoprothrombinemia. Hypoalbuminemia occurs when 1) hepatic production is insufficient to meet demand, either as a result of insufficient production or increased consumption or 2) there is excessive loss of albumin. Insufficient production can occur in animals with chronic severe hepatic disease or as a result of inadequate protein intake, digestion, or absorption. Because bovine albumin has a half-life of 16.5 days and the reserve capacity of hepatic tissue is so great, liver disease must be chronic and severe to result in severe hypoalbuminemia. In the authors’ experience, cattle with chronic debilitating disease of many causes may be hypoalbuminemic with low or normal total protein. If the A:G ratio is low, chronic inflammatory disease should be suspected. In acute and subacute disease, hypoalbuminemia frequently results from loss of albumin. Avenues of albumin loss include the kidney (particularly the glomerulus), the gastrointestinal tract, hemorrhage and exudation. In many instances, loss of albumin may be accompanied by loss of globulin, resulting in panhypoprothrombinemia.

Renal amyloidosis can result in severe albumin loss in the urine due to glomerular damage. In one report, 5 of 6 cattle with amyloidosis had hypoglobulinenia along with hypoalbuminemia. Panhypoprothrombinemia is the rule in protein-losing gastro-enteropathies such as nematode parasitism, paratuberculosis and salmonellosis. Because digestion and absorption may be impaired in these diseases, decreased production due to amino acid deficiency may contribute to the hypoprothrombinemia in chronic cases. Acute hemorrhage results in panhypoprothrombinemia accompanied by anemia.

Hypoglobulinenia is infrequent in cattle except neonates, either as a result of failure of passive transfer of maternal antibody, or severe infection when transferred antibodies are consumed rapidly prior to the efficient production of endogenous antibody by the young calf.

Hepatic tests

The leakage enzymes aspartate transaminase (AST, formerly SGOT), L-iditol (formerly sorbitol) dehydrogenase(IDH), ornithine carbamoyltransferase (OCT), glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) have been used to evaluate the liver. Of these, AST, LDH and IDH are the most popular in the United States. Both AST and LDH are found in a wide variety of tissues, the most important of which are liver and muscle. Muscle damage, especially due to recumbency in cattle, may result in marked increases of both; hence, AST and LDH should be interpreted in conjunction with a liver-specific enzyme (such as GGT), or a muscle-specific enzyme such as creatine kinase (CK) to determine the source of the tissue insult. Usually, high AST or LDH and normal CK indicates liver disease. If serum is allowed to remain on the clot too long or the sample is hemolyzed, the AST and LDH...
may be falsely elevated because both enzymes are found in red blood cells. Because concentrations of these enzymes are high in serum when damaged cell membranes allow their escape from hepatic cytosol, they indicate cell damage, not abnormal hepatic function. In fact, in chronic or slowly progressive hepatic disease, these enzymes may be within or below reference ranges because few hepatocytes are being damaged at one time, or because hepatocellular mass is substantially reduced. Consequently, these enzymes may be more sensitive indicators of acute disease such as some toxicities and infectious hepatitis. They may also be high in cattle with hepatic lipidosis, passive venous congestion and diseases that cause distension of the forestomachs and abomasum. IDH is a specific and sensitive indicator of hepatocellular damage. Unfortunately, its usefulness is limited by its relatively instability in vitro.

The "cholestatic", enzymes gamma glutamyltransferase (GGT) and serum alkaline phosphatase (SAP), are more sensitive to biliary obstruction caused by conditions such as fascioliasis or cholelithiasis. The cholestatic enzymes are more likely to be high in chronic hepatic disease than are the leakage enzymes because fibrosis constricts and blocks some bile ducts.

Although GGT is found in many tissues, the source of essentially all of the GGT in the serum is the biliary and hepatocellular membranes. Therefore, it is one of the most liver-specific tests available to veterinarians. Serum GGT rises principally in cholestatic disease, although hepatocellular diseases in which cholestasis is a secondary feature, also causes an increase in GGT. Because it tends to decrease less rapidly than the other leakage enzymes, it may be of more value in identifying cattle with chronic hepatic disease. Serum GGT of pre-colostral calves is similar to that of mature cattle, but serum concentrations rise sharply following consumption of colostrum, which is rich in GGT. By 24 hours after colostral intake, serum GGT concentration is 50 to 100 times that of colostrum-deprived calves. In fact, serum GGT can be used to estimate the success of passive transfer, but not to detect hepatic disease in neonates.

Serum alkaline phosphatase, a useful indicator of hepatic or cholestatic disease of dogs, is often included in chemistry profiles of cattle. Several isoenzymes from different tissues have been identified, and almost all of the SAP in healthy cattle is of osseous origin. In cattle with hepatic disease, SAP of hepatic origin increases, but the increase is not large in magnitude. Therefore, SAP is of limited diagnostic value for hepatic disease of cattle. Interestingly, though not clearly explainable, SAP was found to be useful as a prognostic indicator in cattle with abomasal volvulus.

Bilirubin is a breakdown product of hemoglobin that is conjugated and excreted by the liver. Unconjugated (or direct) bilirubin is the result of rapid breakdown of hemoglobin which occurs in acute hemolysis. Conjugated (or indirect bilirubin) accumulates in plasma when there is intra- or extrahepatic biliary obstruction. The plasma concentration of bilirubin in healthy cattle is very low compared to that of the other species; and the magnitude of increase is relatively small, even in severe liver disease. Severe bilirubinemia and icterus in cattle is almost always a result of hemolysis, and hence, is primarily due to unconjugated bilirubin.

Though usually considered an index of renal function, blood or serum urea nitrogen (BUN or SUN) is also an indicator of hepatic function. In the liver, ammonia is converted to urea. In severe hepatic failure or partial vascular anomaly, SUN is low while ammonia is high. However, low SUN is not associated only with hepatic disease. Because rumen microbes use urea to synthesize protein, the rumen acts as a "sponge" for urea in cattle that are anorectic or protein-deprived.

Laboratory reference ranges for mature cattle are invalid for neonatal calves, especially those under a week of age. Neonatal calves have somewhat higher concentrations of bilirubin, AST, SAP, and SBA and markedly higher concentrations of GGT than do mature ruminants.

**Electrolytes**

The serum electrolyte profile typically includes sodium (Na), potassium (K), chloride (Cl), and total carbon dioxide (TCO₂) or bicarbonate (HCO₃⁻). From these values, the anion gap (AG) can be calculated. Although there is a nominal difference between the TCO₂ and HCO₃⁻, the HCO₃⁻ usually being slightly smaller, we will consider them equivalent in this paper. Serum electrolytes are useful in the evaluation of several body systems, as well as for the formulation and monitoring of fluid and electrolyte therapy. Due to the abundance of K and scarcity of Na in erythrocytic fluid relative to serum, hemolysis can falsely increase serum K and decrease serum Na in cattle.

Because their concentrations change in concert in a number of conditions, the electrolytes will be discussed together. Sodium is the major extracellular cation, while Cl and HCO₃⁻ are the major extracellular anions. Chloride and HCO₃⁻ often maintain a reciprocal relationship in extracellular fluid. Because the majority of the exchangeable Na and Cl are found in the extracellular fluid, measuring serum Na and Cl provides an accurate assessment of the total body status of these electrolytes. Serum potassium, on the other hand, provides a less reliable and sometimes paradoxical reflection of total body K status because only a small portion (approximately 5%) of the animal’s K is in the extracellular fluid. Changes in blood pH greatly affect serum K by causing the movement of K across cell membranes; K moves into cells during alkalosis and out of cells during acidosis. Therefore, serum K should be interpreted along with serum HCO₃⁻. Serum HCO₃⁻ is a measure of metabolic acid-base balance; concentrations above the reference range indicating metabolic alkalosis and those below indicating metabolic acidosis.

Hypernatremia and hyperchloremia occur in salt toxicity/water deprivation, but are not commonly present in cases of dehydration because typically fluid loss in cattle occurs with concurrent loss of electrolytes. Cattle with clinical "salt toxicity" may have normal
serum Na because clinical signs often do not occur until after the cattle drink, and serum Na concentration and osmolality return to normal. Hyperkalemia is almost always secondary to acidosis as K moves out of the intracellular fluid into the extracellular fluid. Therefore, serum K is an unreliable index of total body K. For example, diarrheic calves often are acidic and hyperkalemic, but they have total-body K depletion because of fecal K loss. In these cases, as in most cases where serum K is increased secondarily to acidosis, K supplementation may be indicated during or immediately following correction of acidosis. Hypochloremia, hypokalemia, metabolic alkalosis and, to a lesser degree, hyponatremia, are typical findings in obstructive gastrointestinal diseases including abomasal volvulus, displaced abomasum, vagal indigestion, intussusception and cecal torsion. In these diseases, HCl is sequestered in the abomasum, causing hypochloremia, metabolic alkalosis, and secondary hypokalemia. In general, the more orad the lesion (abomasal impaction vs jejunal intussusception), and the more complete the obstruction (abomasal volvulus vs LDA), the more severe the alkalosis and hypochloremia. Hypochloremia and metabolic alkalosis are fairly non-specific abnormalities in sick cattle however. In a study of over 500 mature cattle in the authors' hospital, over 40% of the dehydrated cattle were hypochloremic and/or severe the alkalosis and hypochloremia. Hypochloremia and metabolic alkalosis are fairly non-specific abnormalities in sick cattle.

Endogenous and exogenous steroids increase gluconeogenesis and increase serum glucose. Xylazine causes a dose dependent dextrose, even though hypocalcemia prevents the release of insulin from the islet cells of the pancreas resulting in hyperglycemia. Following administration of dextrose solution, xylazine, or corticosteroids. It is interesting that most milk fever remedies contain fluoride-containing tubes should be used if timely separation is not possible. Hyperglycemia occurs in stress, milk fever, and following administration of dextrose solution, xylazine, or corticosteroids. It is interesting that most milk fever remedies contain dextrose, even though hypocalcemia prevents the release of insulin from the islet cells of the pancreas resulting in hyperglycemia. Endogenous and exogenous steroids increase gluconeogenesis and increase serum glucose. Xylazine causes a dose dependant hyperglycemia that persists for over 6 hours. Diabetes mellitus although uncommon in cattle, causes permanent hyperglycemia if untreated.

Renal tests

Elimination of nitrogenous wastes, such as urea and creatinine (Cr), and concentration of urine to conserve body water are two of the many vital functions performed by the kidney. Evaluation of these functions is exploited in the diagnosis of renal disease. Serum or blood urea nitrogen (SUN or BUN) and serum creatinine (Cr) are rough indices of glomerular filtration rate. The generous reserve capacity of the kidney makes SUN and Cr insensitive indicators of renal function; 75% loss of functional renal mass is required for azotemia to occur. Slightly more sensitive than SUN and Cr, the urinary specific gravity (USG) can detect about a 67% loss of functional renal tissue. The USG is most easily estimated by refractometry. By convention, USG of ≥ 1.025 is considered indicative of appropriate concentrating ability in the face of dehydration or azotemia. It is quite common, however, for normally hydrated cattle, especially dairy cattle, to have USG <1.025.

Azotemia, the accumulation of nitrogenous wastes in the blood, is reflected in the serum chemistry profile as high SUN and Cr. Remember - AZOTEMIA DOES NOT EQUAL RENAL DISEASE! Azotemia can be classified as renal (due to renal disease), prerenal (due to sluggish renal blood flow, as in shock or dehydration), or postrenal (due to obstruction of urine outflow, as in urolithiasis). Though not without exception, the simplest way to distinguish among the three is by measuring the USG. In azotemic cattle, if the USG is ≤ 1.025, the azotemia is prerenal; if the USG is >1.025, the azotemia is renal. In postrenal azotemia, urine is often difficult or impossible to obtain, and the diagnosis is based on physical examination. In our experience, cattle with prerenal azotemia eliminate urea and Cr rapidly when rehydrated, often returning to or near the reference range in 24-48 hours if appropriate fluid therapy and correction of the primary problem is accomplished.

Urea is formed in the liver by the detoxification of ammonia, a by product of protein metabolism. Therefore SUN is influenced by diet and hepatic function. Urea is recycled in a functional rumen, a process which may tend to moderate the rise in SUN in renal disease and result in a low SUN/Cr ratio. Although Cr, a product of energy metabolism in muscle, can be very low in emaciated cattle with little muscle mass, it tends to be less influenced by extraneous factors than SUN. For this reason, Cr is the test of choice over SUN.

While SUN, Cr and USG can identify renal disease, the final diagnosis cannot be obtained from this information. For example, acorn toxicity, pyelonephritis, and amyloidosis are diseases which cause renal failure in cattle. These diseases cannot be distinguished from one another simply based on the results of the chemistry profile. However, the characteristics of the urine in each of these diseases is very different. Whenever renal disease is suspected, a complete urinalysis should be performed, as well as, rectal palpation of the kidneys. Ultrasonography and renal biopsy may also be informative.

Glucose

Glucose metabolism is unique in ruminants because they absorb essentially no pre-formed glucose from the gut. The reference range for serum glucose in adult cattle is lower than for calves and non-ruminant species. Erythrocytes metabolize glucose in vitro in a blood tube at a rate of about 10% per hour at room temperature. Serum should be separated from the clot within 30 minutes, or sodium fluoride-containing tubes should be used if timely separation is not possible. Hyperglycemia occurs in stress, milk fever, and following administration of dextrose solution, xylazine, or corticosteroids. It is interesting that most milk fever remedies contain dextrose, even though hypocalcemia prevents the release of insulin from the islet cells of the pancreas resulting in hyperglycemia. Endogenous and exogenous steroids increase gluconeogenesis and increase serum glucose. Xylazine causes a dose dependant hyperglycemia that persists for over 6 hours. Diabetes mellitus although uncommon in cattle, causes permanent hyperglycemia if untreated.
Muscle enzymes
As previously mentioned, LDH and AST are released into plasma as a result of muscle damage, but they are not muscle-specific enzymes. Serum CK, on the other hand, is a very sensitive and specific indicator of muscle damage. Subtle increases can occur due to intramuscular injection, exercise or struggling. Recumbent mature cattle may have >100-fold increases due to the secondary pressure damage that is a part of the downer cow syndrome. Very high concentrations of CK in the absence of recumbency, or in young recumbent cattle suggest primary myopathy, such as white muscle disease or Senna toxicity. The half-life of CK in serum is short, and CK concentrations fall rapidly in recumbent animals even if they remain recumbent. Because AST concentration rises and falls more slowly, it can be used in combination with CK to stage muscle damage. In recumbent cattle, if the AST is very high and the CK is not, the damage is likely several days old. In an attempt to use laboratory tests for prognosis, New Zealand investigators found that fewer than 5% of cows with an AST value > 7.4 times the upper limit of the reference range survived. For CK, the "critical" value above the reference range was related to the duration of recumbency (Table 2).