

RUMEN FLUID ANALYSIS

PURPOSE

Rumen fluid collection and analysis is a diagnostic aid that is beneficial for individual animals exhibiting abdominal distension, reduced fecal output, anorexia, and abnormal rumen texture or motility, and it can be used for herd-level monitoring for subacute rumen acidosis.

COLLECTION

Orogastric/Nasogastric tubing

- Orogastric intubation is achieved using a 2-3 meter, >1cm internal diameter stomach tube passed through a Frick or similar oral speculum
- Alternatively, nasogastric intubation is easily performed in ruminants by passage of the tube through the ventral nasal meatus; this method can be less stressful than orogastric tubing and obviates the need for an assistant to hold the speculum while the sample is collected
- The fiber mat of the rumen may limit the passage of the tube into the ventral fluid portion of the rumen; weighted tubes may be used to improve passage through the fiber mat
- This method carries increased risk of saliva contamination, which may significantly alter pH, methylene blue reduction time and electrolyte concentrations in the rumen fluid sample
 - Animals which struggle excessively during passage of the tube are more likely to have salivary contamination
 - Collecting a large volume of rumen fluid reduces the overall effect of saliva on sample pH
- Once the tube is located in the ventral rumen sac, a dosing syringe can be used to aspirate fluid into the tube
- In cattle with a rumen fistula, fluid can be easily collected through the animal's side (see Rumen Transfaunation for details of technique)

Ruminocentesis

- Eliminates risk of salivary contamination
- Preferred sampling method for rumen pH monitoring for subacute rumen acidosis
- Potential complications include subcutaneous abscessation, localized peritonitis, abomasocentesis if the abomasum is left displaced, puncture of the late gestation uterus and the inability to obtain a large sample volume
- Procedure:
 - Locate an area in the lower left ventrolateral abdominal quadrant, cranial and horizontal to the stifle, and 8 inches caudal to the last rib in the adult bovine
 - Clip and aseptically prepare the area
 - Hobble the animal or have an assistant lift the tail upward ('tail jack')
 - Thrust a 16 gauge, 5 inch needle attached to a 12 or 20 mL syringe quickly and directly into ventral ruminal sac and withdraw a volume of fluid
 - Small pieces of fiber are easily pulled against the bevel of the needle, preventing sample collection; alternating gentle aspiration, release and positive pressure can be used to obtain sufficient sample volume for fluid analysis

ANALYSIS AND INTERPRETATION

- **Color**
 - Diet influence
 - Corn silage or straw creates yellow- brown fluid
 - Concentrates create olive- brown color
 - Pasture creates green color
 - Pathology influence
 - Prolonged stasis or putrefaction creates a black green color
 - Lactic acidosis creates a milky grey color
- **Odor**
 - Normally aromatic and inoffensive
 - Acidic or sour odor can indicate lactic acidosis
 - Rotting odor can indicate rumen putrefaction
 - Ammonia odor can indicate urea toxicity
- **Consistency**
 - Normally slightly viscous
 - Increased viscosity can occur with saliva contamination
 - Reduced viscosity with few feed particles suggests anorexia and poor microbial activity
 - Stable bubbles or foam can reflect frothy bloat
 - Milk clots can be observed in nursing calves with abomasal reflux or esophageal groove failure
- **pH**
 - May be measured with pH paper or portable pH meter
 - For subacute rumen acidosis monitoring, rumen pH is best measured 2-4 hours after feeding concentrates or 4-8 hours after feeding a total mixed ration due to differing rates of fermentation and absorption
 - Reference ranges
 - Grass-fed: pH 6-7
 - High grain diet (adapted): pH 5.5-6.0
 - Abnormal rumen pH interpretations (interpreted based on known diet)
 - 5.5-6.0 in pasture fed cattle, indicates early lactic acidosis
 - < 5.5 indicates lactic acidosis, except that some well-adapted feedlot animals may have rumen pH of 5.0
 - 7-8 can occur with reduced feed intake for more than two days; simple indigestion
 - >8.0 suggests saliva contamination of sample, rumen putrefaction from prolonged rumen stasis, or urea toxicity
 - An animal with lactic acidosis may have a normal rumen fluid pH if anorexia has been prolonged due to continued saliva production
- **Microscopic examination**
 - Place one drop of fresh rumen fluid on a warm microscope slide and observe unstained under low power (10x)
 - The sample may then be stained as described below to evaluate bacterial populations
 - **Protozoa**

- Normal rumen fluid contains > 40 protozoa per low power field
- There should be three groups of protozoa visible: small, medium and large, and they should be rapidly moving
- It may be important to identify a size-category of protozoa which is missing; the small group tends to be more resistant to pH values of <6; i.e. larger protozoa die off earlier in rumen acidosis
- Staining with Lugol's iodine may help with assessing the health of protozoa - healthy protozoa with adequate energy stores uniformly stain with iodine, whereas protozoa starved for nutrition do not stain well
- **Bacteria**
 - Gram stain of rumen fluid reveals the populations of bacteria present
 - Normal rumen fluid contains varied populations of predominantly gram-negative bacteria
 - Lactic acidosis results in a more homogenous, predominantly gram-positive population indicator
 - It is important to remember that Gram staining was developed for identification of single-population bacteria derived from culture colonies; it is therefore difficult to use Gram staining to evaluate the ratio of Gram positive to negative populations, as staining may not perform as originally intended and many non-bacterial organisms will take up stain
- ***Sedimentation time***
 - Also called the sedimentation activity time or sedimentation/flotation test
 - Evaluates fermentative activity of rumen flora
 - Place fresh fluid into a clear tube immediately after collection and allow the fluid to sit, observing the time required for complete sedimentation of solid particles
 - Smaller particles sink, larger particles float on the bubbles of fermentation initially and then as flora die, sedimentation will occur as fermentation ceases
 - Reference range is 4-8 minutes for complete sedimentation
 - Very active fluid may exhibit sedimentation of fine particles with subsequent flotation
 - Inactive fluid shows rapid sedimentation with little to no flotation, due to lack of fermentative gases
 - Rumen acidosis, prolonged anorexia, indigestible feeds inhibit flora activity
 - Stable froth presence indicates frothy bloat or some types of vagal indigestion
 - May be no sedimentation or flotation
- ***Redox potential/Methylene blue reduction test***
 - Evaluates degree of bacterial fermentation (reducing capacity) by rumen flora
 - Procedure:
 - Mix 10 mL of fresh rumen fluid + 0.5 mL of a 0.03% solution of methylene blue in a test tube
 - Allow to stand and determine time taken to decolorize sample until only a blue ring remains at the top of the sample; it can be useful to have a second tube of rumen fluid with no methylene blue added to help determine when color has returned to that of the initial sample (this second sample can also serve as the sedimentation time sample)

- Reference range for cattle: 2-6 minutes; concentrate diets are associated with the faster times in that range, forage diets with the slower times
- Decolorization which takes longer than 10-15 minutes indicates inadequate anaerobic bacterial population
- It should be noted that sheep have faster clearance times (1-4 minutes) and that testing is invalid with samples with a pH < 5.5
- **Chloride concentration**
 - Useful to localize site of obstruction in animals with rumen distension
 - Elevated rumen chloride indicates abomasal reflux of hydrochloric acid into the rumen, localizing obstruction or ileus at or distal to the pylorus
 - Generally these animals are also hypochloremic and have metabolic alkalosis
 - Elevated values cannot be interpreted when oral electrolyte solutions have been administered
 - Reflux of abomasal hydrochloric acid will not alter rumen pH, making rumen chloride a more sensitive test for reflux
 - This test may be run by most reference labs on the supernatant of a centrifuged rumen fluid sample
 - Request that the sample be run by the same method as a urine chemistry
 - Relatively stable over time: Can be stored at room temperature up to 9 hours and refrigerated for 24 hours and still be considered accurate
 - Test should be run prior to oral fluid therapy to eliminate sample bias due to chloride-containing salt administration
 - Reference values in cattle are <30 mEq/L (10-25 mEq/L) and in sheep < 15 mEq/L
- **Miscellaneous tests**
 - The following tests of rumen fluid are generally limited in their clinical utility and are not frequently performed in clinical practice
 - Rumen osmolality
 - Cellulose digestion test
 - Glucose fermentation
 - Nitrite reduction
 - Total titratable acidity
 - Fatty acid concentrations
 - Lactic acid concentrations
 - Ammonia concentrations



MISCELLANEOUS

Suggested Reading

Alonso AN. Diagnostic analysis of rumen fluid. *Vet Clin North Am Food Anim Pract* 1979; 1:363-376.

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