Ruminal Fluid Examination

Microbes in the reticulorumen include bacteria, protozoa, fungi. Bacteria, along with protozoa, are the predominant microbes and by mass account for 40-60% of total microbial matter in the rumen. They are categorized into several functional groups, such as fibrolytic, amylolytic, and proteolytic types, which preferentially digest structural carbohydrates, non-structural carbohydrates, and protein, respectively. Protozoa (40-60% of microbial mass) derive their nutrients through phagocytosis of other microbes, and degrade and digest feed carbohydrates, especially starch and sugars, and protein. Although protozoa are not essential for rumen functioning, their presence has pronounced effects. Ruminal fungi make up only 5-10% of microbes and are absent on diets poor in fibre. Despite their low numbers, the fungi still occupy an important niche in the rumen because they hydrolyse some ester linkages between lignin and hemicellulose or cellulose, and help break down digesta particles.

☯Indications☯

1. It is often essential to establish an accurate diagnosis of diseases of the rumen.
2. It is also essential when rumen fluid is collected for therapeutic transfaunation.

☯Methods of collection☯

1. Needle puncture of the rumen.
2. Oral or nasal passage of a collection tube is preferred to avoid risk of peritoneal contamination from needle puncture. also avoid continuous suction but suction is done with 10 minute interval to take representative sample.
3. Manual method from slaughtered animal

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☯Examination of rumen fluid☯

General remarks:-

1. The sample should be evaluated as soon as possible after collection to minimize the effects of cooling and air exposure on protozoal activity and pH.
2. Estimation of biochemical characters can be delayed to 9 hours in room temperature sample and up to 24 hrs on a refrigerated sample.
3. Transportation of rumen fluid for long distances must be done through double Jacket container (Coleman).

(A) Physical characters

1. Color:

A. Normal color varies depending on the nature of feed:

I. Yellowish brown color ���. Grain ,silage ration ,Hay ration.
II. Deeper green color ���. Green ration

B. Abnormal

I. Milky gray ���. Grain overfeeding
II. Darker greenish ���� Prolonged ruminal stasis and/or decomposition of rumen contents.(protein putrefaction)
III. Gray with clots of milk ���Calves with abomasal reflux or oesophegeal groove failure

2. Consistency

A. Normal

I. Slightly viscous consistency

B. Abnormal

I. Watery ���. Inactive bacteria and protozoa
II. Excess frothy ���Frothy bloat from primary tympany or vagus indigestion.

3. Odor

A. Normal

I. Aromatic acceptable odour

B. Abnormal

I. Ammonia smell ���Urea poisoning
II. Moldy rotting odor(putrified) ���. Protein putrefaction
III. Intensely sour odor ���. Excess lactic acid production from grain or carbohydrate overfeeding.

4. pH

- It is measured by universal pH papers indicators or by pH meters. It must be measured immediately after sampling.
- Normal pH differ acc to type of food as ranges between 6- 7in animals on a mostly forage diet but is lower at 5.5 - 6.5 in animals fed mostly grain.

- Also ph differ according to type of sampling : immediately after feeding …acidic
  After certain period……...alkaline

Elevated pH (Rumen alkalosis)8-10:

- Simple indigestion.
- Urea indigestion.
- Putrefaction of rumen ingesta.

Lowered pH (Rumen acidosis)

- Engorgement with readily digestible carbohydrates.
- Chronic rumen acidosis (pH 5 - 5.5).
- Abomasal reflux from abomasal disease, vagal indigestion and intestinal obstruction.

(B) Chemical characters

1. Sedimentation activity test.

- It provides a rapid evaluation of protozoa activity.
- Must be conducted just after sample collection.

Technique.

1. Put a sample of rumen fluid in a test tube and let to stand.
2. Measure the time needed for completion of sedimentation of fine particles and floatation of coarse solid particles

- Normal time is 3 - 9 minutes.
- Abnormal time:

a-prolonged time …indicate protozoa in activity

b-Very rapid sedimentation with no floatation occurs in :-

o Rumen acidosis.
o Prolonged anorexia.
o Inactive microflora from indigestible roughages.

c-No appreciable sedimentation or floatation :-

o Frothy bloat.
o Some cases of vagal indigestion.

2. Methylene blue reduction test

- It reflects the anaerobic fermentative metabolism of the bacterial population.

Technique.

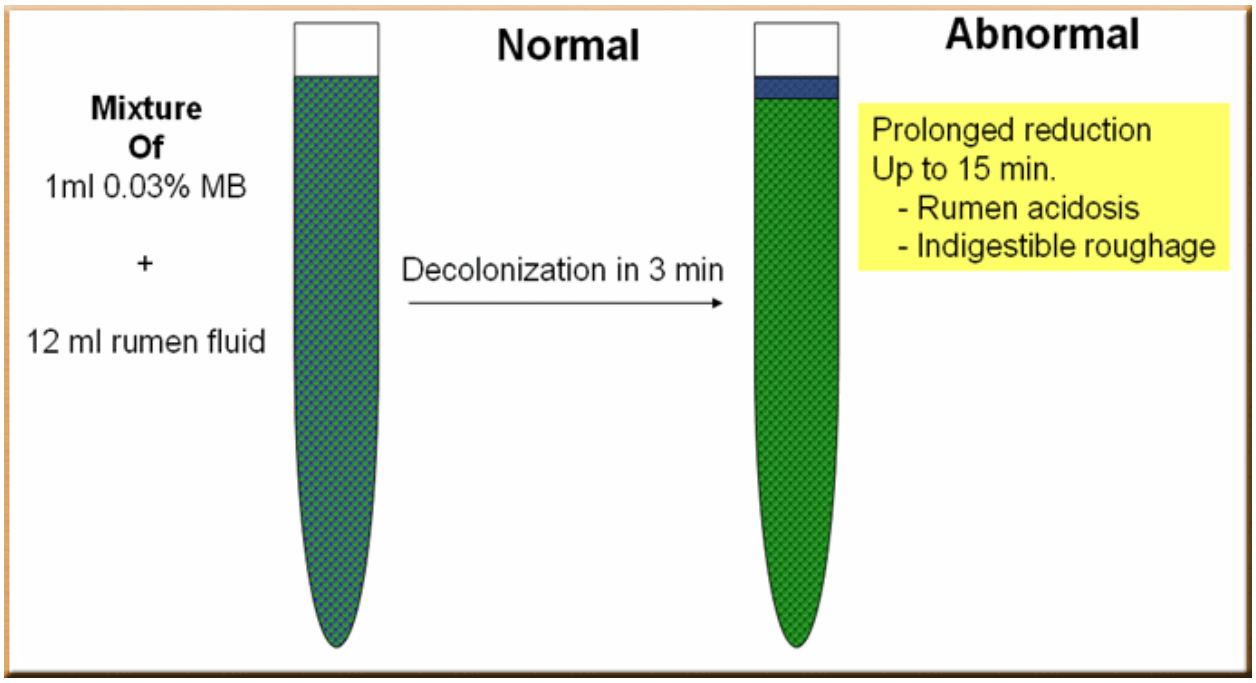
Mix 20 ml ofrumen fluid with 1 ml of 0.03 % methylene blue in a test tube and let to stand at room temperature.

Measure the time needed for the color of the mixture to be changed.

Normal rumen fluid from cattle fed on a hay and grain diet needs 3 mm. to decolorize leaving a narrow ring of blue color at the top of decolorizing mixture.

Abnormal reduction of time up to 15 M indicates:

- Indigestible roughage..
- Rumen acidosis.



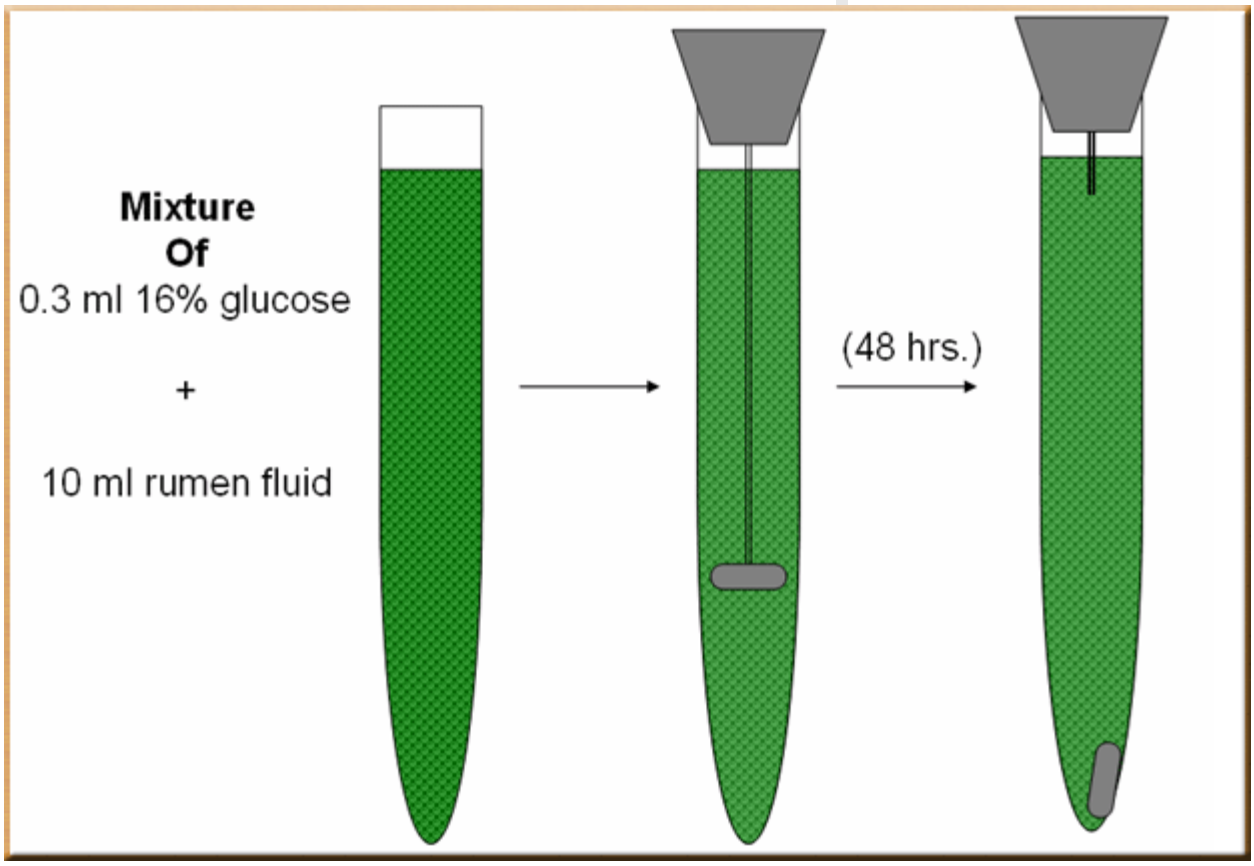
3. Cellulose digestion test.

depend in the action of cellulitic bacteria

Technique.

- Mix 10 ml of rumen fluid with 0.3 ml of 16 % glucose * Read solution in a test tube.

- Immerse a thread of pure cellulose. The lower end is weighted by a glass bead.
- Incubate the tube at 37 c.
- Record the time for the bead to be dropped free at the bottom of the tube.
- Interpretation
  - A fully active rumen fluid will digest the cellulose foaming. within 24 - 30 hours.
  - The test takes a long time and is not very accurate.



- 3-microscopic examination
  - 1-protozoa(microfuna)

- A. Motility
- Is examined in a fresh film under low power (X 40 to X 100) magnifying microscope.
- Motility is judged as follows:
- +++ ⟶ Highly motile and very crowded.
- ++ ⟶ Motile and crowded.
- + ⟶ Sluggish motility and low number.,
- 0 ⟶ No or sporadic alive protozoa
- Normal fluid ⟶ Fauna are motile

- Abnormal fluid ⟶ Sluggish or no movement at all recent acidosis.

B. Classification of various types of fauna.

- Is made in fresh fixed rumen fluid film.
- The film is stained with Lugols iodine.
- Classification depends upon size, morphologic characters as number and site of flagellae, presence of vacuoles macro and micronucleus, skeletal plates and various pines and projections.

(D) Bacteria

1. Groups, based on function include those that breakdown cellulose, starch and sugar and those that form methane,proteolytic bacteria and other groups.
2. An air dried smear of rumen fluid is stained by Grams stain, Iodine, PAS, nigrosine, Congo red or others are used for bacteria identification.
3. Examine as follow:

   *make filtration to the ruminal fluid

   *then make centrifugation to the filtrate

   *discard the supernatant and take the sediment

   *stain sediment with gram stain then examined microscopically

   normal ⟶ gram negative bacteria (red color)

   abnormal ⟶ gram positive bacteria (violent color)

(E) Fungi

1. Are present as yeasts (e.g. Candida sp.)
2. Such micro-organisms are sometimes present in exceptional numbers during rumen acidosis.

Examine as follow :

Drop from supernatant with drop of iodine

Examine under microscope