Rumen Fluid Examination

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Indigestion in cattle constitutes one of the most important problems which causes great economic losses to Indian farmers. Although indigestion is a very common complaint in veterinary practice, it represents a challenging diagnostic problem, because of the non-specific nature of its manifestations of clinical signs. In day to day practice many diagnosis of various diseases would be difficult without the assistance of various examinations such as those involving milk, blood, urine or dung etc., of the sick animals. It is the rumen with all its varied activities that makes a ruminant different from other animals. It is logical therefore to check the physical and chemical properties of the ruminal content, when one is confronted with an anomaly of unknown cause in these animals.

Examination of rumen fluid was introduced for clinical diagnosis during 1950. Some of the tests described below are easy to carry out in day to day practice and they enable biochemical disorders of fore-stomach digestion to be confirmed or excluded. For this purpose sufficient rumen fluid can be collected easily from the sick animals by using RUMEN FLUID EXTRATION PUMP.

Rumen fluid extraction pump [for cattle and Sheep and Goat] consists of :-

1. A specially designed suction strainer,
2. Three meter nylon tube with perforation at the tip which is covered by a stainless steel spiral sound,
3. A suction pump,
4. An air tight sampling bottle with two way 'T' joint.

Use of Rumen fluid extraction pump

1. To diagnose various digestive and toxic problems in ruminants,
2. To evacuate the rumen contents in various diseases [Rumen acidosis etc.,] as treatment to save the animals,
3. To collect Rumen contents from healthy animals for cud transplantation to sick animals to revive the normal flora and to young calves for quick and healthy growth.

Limitation

If the animal has been starving for considerable time and the rumen content is semisolid in nature, rumen fluid may not be obtained by using the rumen fluid extraction pump. In such cases, physiological saline is to be pumped in and massaged well before the collection.
Preparation of the instrument for collection of Rumen Fluid

1. Unscrew the suction strainer and see that the perforations at the end of the nylon tube are clean. If there is any blockage it should be cleaned. Also make sure that the full length of the nylon perforated end is projecting out.

2. Check the suction pump for its working condition. The washer may be damaged due to shrinkage or brittleness if it is not used regularly. So, to overcome this problem, remove the piston from the suction pump and widen the leather washer by hand and apply either grease or oil.

3. Fix the two way 'T' joint tightly to the sampling bottle to prevent the air leakage.

4. Fix the nylon tube from the suction strainer into the vertical end of the two way 'T' joint. Horizontal end of the 'T' joint should be connected to the suction pump. If they are not properly fixed, rumen fluid cannot be collected.

5. Check the entire set by dipping the suction strainer into a bucket of water and operating the suction pump.

6. Apply liquid paraffin over the stainless steel spiral sound of the instrument which is ready for collection.

IMPORTANT POINTS TO BE OBSERVED BEFORE AND AFTER THE COLLECTION OF RUMEN FLUID

1. About 200ml of Rumen fluid sample is to be collected for various tests.
2. The Rumen fluid can be kept in room temperature for 9 hours and in refrigerator for 24 hrs.
3. After collection, rumen fluid container should be immediately closed air tight.
4. During collection discard the first collection of Rumen fluid and then collect the sample so that error in pH can be minimised.

COLLECTION PROCEDURE:

Cattle

Restrain the animal with nose grips. Open the mouth by pulling out the tongue to one side. Hold the head of the animal high and intubate (introduce) the stainless steel spiral sound with suction strainer until it reaches the rumen. Collect the rumen fluid by operating the suction pump.
Sheep and Goat

Restrain the animal in between the knees of an Attendant at the level of the forelimbs. Then instruct the attendant to raise the head above. Insert the mouth gag across the mouth and intubate the Rumen fluid extraction pump by introducing suction strainer through the hole in the mouth gag. Pass over the tongue and past the epiglottis into the cranial part of the oesophagus. At this level, care should be taken not to enter into the trachea and cause any injury. Once you make sure that the suction strainer is in the oesophagus, pass further down the oesophagus until it reaches the rumen. You can also visualise the tube passing through the oesophagus on the left side of the neck of the animal. When the suction strainer is dipped into the rumen contents, operate the suction pump and collect the required quantity of rumen fluid.

AFTER USE

For longevity and efficient usage of this instrument clean the suction strainer with suction line and collection bottle in running water without any rumen contents and dry it before packing.

The common tests that can be conducted in the Rumen Fluid to pinpoint the correct diagnosis:

<table>
<thead>
<tr>
<th>Colour</th>
<th>sedimentation activity time (SAT) or Stratification</th>
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<td>The common tests that can be conducted in the Rumen Fluid to pinpoint the correct diagnosis:</td>
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- Colour
- Odour
- Consistency
- Sedimentation activity time (SAT) or Stratification
- pH
- Cellulose digestion test
- Glucose fermentation test
- Redox potential or Methylene blue reduction test (MBR Test)
- Volatile Fatty acid, lactic acid,
- Total Acidity (Titratatable acidity)
- Chloride
- Protozoa
- Bacteria

Examination of Rumen Fluid sample

Take 100 ml of Rumen fluid in a beaker and conduct the following Bio-physical examination (A to D) in well lighted area:

A) Colour

Normal Colours

- Pure green - in grazing
- Yellowish brown - in straw feeding
- grey/brownish green - concentrate and straw feeding
Abnormal colours

- Dark brown/Dark green - Simple inactivity of flora and fauna, Rumen acidosis.
- Slightly milky - Chronic rumen acidosis.
- Milky green - Acute rumen acidosis
- Dark Green - Hydrochloric acidosis
- Greenish black - Vagus indigestion, decomposition of food

B) Odour:

Normal: Aromatic

Abnormal:

- Stale/indifferent - inactive rumen juice
- Acrid (acid) - Lactacidosis/hydrochloric acidosis/Pyloric obstruction
- Foul/Putrid - Protein overfeeding
- Slightly ammoniacal - rumen alkalosis (urea poisoning)
- Musty or faecal - Vagus indigestion

C) Consistency

- Slightly viscous - Normal
- Extremely viscous - Saliva mixed
- Watery - inactive rumen fluid
- Foaming - abomasal dilatation or frothy bloat
- Mixture of watery and foam - rumen decomposition (E. Coli, proteus)
- Slimy pulp - Overfeeding
- Semiliquid - Vagus indigestion

D) Stratification or Sedimentation Activity Time (SAT)

100 ml freshly collected rumen contents, filtered through gauze if necessary is observed as it settles in a glass cylinder.

In Normal Animal:

- Fine food particles and infusoria - begin to settle at once.
- Larger and more fibrous particles - carried upward forming broad upper layer.

The above process of complete sedimentation and flotation - takes 4 to 8 minutes.

In abnormal Cases:

- Rapid sedimentation and absent or retarded flotation - Inappetence, starvation and feed without nutritive value

- Rapid floatation with abundant foam and solid components remain in suspension for long time - Decomposition in rumen

- Absence of solid particles and gas bubbles - Acute rumen acidosis

- Hence no sedimentation and floatation - Traumatic reticulo peritonitis

- Pulpy to firm above and fluid below - Vagus indigestion

Absence of stratification
E) **Hydrogen ion concentration (pH)**

Should be measured immediately after collection without much exposure to atmospheric air. It can be measured by indicator paper in field conditions.

**Significance**

- **Physiological variation**: 5.5—7.0
- **Ration rich in crude fibre and/or protein**: Higher range 6.0-6.8
- **Ration rich in starch or sugar**: Lower range [5.5-6.0]

**After starvation for 24 hrs. or more**

- **Urea poisoning**: Upto 8.5
- **Rumen decomposition**: Upto 8.5

**Hydrochloric acidosis**

4.3-7.0 [average 6.3]

**Rumen acidosis**

4.0-5.5 Even may reach less than 4.00 in acute cases.

F. **Cellulose digestion test: (CDT)**

Take 10 ml of strained rumen fluid and add 0.3 ml of 16% glucose solution in a capped test tube. Suspend a thread of pure cellulose (free from any synthetic fibres) or single strand of unmercerized cotton thread in the rumen fluid. The lower end of the cotton thread is tied with a glass bead or other weight, which must immerse in Rumen fluid. Then tightly close the test tube and incubate at body temperature (39°C either in incubator or near a light bulb).

**Significance**

Normally, digestion of cellulose takes place within 48-54 hrs. So, in fully active rumen fluid the weight in the lower end of cotton thread will fall to the bottom of the tube within that time due to digestion of cotton thread. If the thread has not broken within the normal time it should be interpreted as cellulose digestion time is being delayed due to inactive rumen fluid.

G. **Glucose fermentation test**

It is performed in a fermentation saccharometer. Take 10 ml of rumen fluid and add 0.5 ml of 16% glucose solution in the saccharometer and keep at 39°C. The result is read after 30-60 minutes.

**Significance**

Normal rumen fluid containing active microflora will ferment the glucose and result in formation of gas.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tbody>
<tr>
<td>1-2 ml gas/hr</td>
<td>Rumen fluid containing active microflora</td>
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<tr>
<td>No gas</td>
<td>Rumen fluid containing inactive microflora and acute rumen acidosis.</td>
</tr>
<tr>
<td>Decreased gas</td>
<td>Rumen decomposition, rumen alkalosis, acute rumen acidosis, hydrochloric acidosis.</td>
</tr>
<tr>
<td>Normal/increased gas</td>
<td>Latent rumen acidosis</td>
</tr>
<tr>
<td>Increased gas</td>
<td>Foamy bloat</td>
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</tbody>
</table>

**H) Redox potential or methylene blue reduction time (MBR Test)**

This is measured by using a redox dye, methylene blue. Take 20 ml of freshly collected rumen fluid and add 1 ml of 0.03% methylene blue solution and mix. Measure the time required for decolouration of the sample using a plain rumen fluid as a basis for comparison.

**Significance**

It is one of the most reliable test to assess the microbial status of rumen fluid.

- **In normally active microflora**
  - Methylene blue reduced within 3 minutes,
  - 3-6 minutes
  - More than 15 minutes,

- **Inactive flora due to**
  - Ration poor in structure

- **Inappetence**
  - Rumen acidosis
  - Less than 5 min above pH 5.2
  - More than 5 min, below pH 5.2

- **Hydrochloric acidosis**
  - More than 5 min

**I) Nitrite reduction**

10 ml of strained rumen fluid is placed into each of 3 test tubes and 0.2 (Tubel), 0.5 (tube 2) or 0.7 (tube 3) ml of 0.025% Potassium nitrite solution is added and kept in water bath at 39°C. Every 5 minutes 1 drop from each tube is placed in the small wells of a ceramic plate and to each drop is added 2 drops each of reagent I (2 ml of sulfanilic acid in 30% acetic acid to make 200 ml), and reagent II (0.6 ml alphanaphthylamine, 16 ml concentrate acetic acid, 140 ml distilled water). Until the disappearance of red colour which will provide information on the activity of microbes that degrade and synthesize nitrogenous compounds. The presence of red colour indicates that still nitrite is present.
Significance

Rumen fluid from cattle fed with mixed ration — Nitrite should disappear in 5-10 minutes-tube 1
Rumen alkalosis, Green fodder, 20 minutes-tube 2
Ruminal decomposition, Bloat 30 minutes-tube 3
Lack of appetite, deficient ration Rapid reduction in all tubes.
Hydrochloric acidosis Slow reduction in a tube
Acute lactacidosis No notable reduction even after 46 minutes.

J. VOLATILE FATTY ACIDS (V F A)

For every 20 ml of rumen fluid, 1 ml of saturated mercuric chloride solution is added and sent to the laboratory for determining total and individual fatty acids.

Significance

Normal:
Total V FA concentration — 60-120 mol/litre of rumen fluid
Propionic acid — 20-25 mol%
Acetic acid — 50-65 mol%
Butyric acid — 10-20 mol%
Formic, valeric, caproic and high fatty acids — 5 mol%

V FA concentration increased on concentrate ration and also 3-5 hours after feeding.
V FA concentration decreased on completion of fermentation (pH increased).

Abnormal:
Loss of appetite, Ration poor in structure. Digestive disorders Total V FA decreased
Increasing amounts of easily digestible carbohydrate — Proportion of individual acids change

K. CHLORIDE

To 0.1 ml of the chloride standard solution, add deionized water 1 ml and put 0.2 ml indicator. Then titrate the standard with mercuric nitrate solution. The end point is slight, but permanent violet colour. This will be the standard. Repeat the same with rumen liquor sample.

Calculation

\[
\text{Test reading} \times \frac{\text{Volume (ml) of 30% HCl solution} \times 100}{\text{Volume (ml) of test sample}} = \text{m.eq.} \times 10^{-1}
\]

Standard reading

\[
\text{Volume (ml) of 30% HCl solution} \times \text{m.eq.} \times 10^{-1} = \text{m.eq.}
\]
This is useful in distinguishing hydrochloric acidosis from lactic acidosis.

Normal level of chloride content in rumen fluid is 15-25 mVal/litre.

**Abnormal:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactacidosis</td>
<td>Less than 40 mVal/litre.</td>
</tr>
<tr>
<td>Hydrochloric acidosis</td>
<td>More than 25-30 mVal/litre.</td>
</tr>
</tbody>
</table>

May increase to 30-100 mVal/litre in:

- a) reflux of abomasal content as a result of obstruction
- b) supplementation of ration with sodium chloride
- c) functional or anatomical pyloric stenosis
- d) abomasitis
- e) abomasal ulcer
- f) sand in stomach or intestine
- g) abomasal displacement
- h) cellulitis of mesentery at its attachment to the abomasum
- i) Paralytic ileus

**L. TOTAL ACIDITY (TITRATABLE ACIDITY):**

One or 2 drops of phenolphthalein are added to 10 ml of rumen fluid and the mixture is titrated with N/10 NaOH until it becomes flesh coloured. The volume of NaOH required (in ml) multiplied by 10 gives clinical units of total acidity:

- Normal rumen fluid: 0-2 clinical units
- Slight acidosis: 2-5 clinical units
- Moderate acidosis: 5-8 clinical units
- Severe acidosis: Above 8 clinical units

**Significance:**

- In hyperacidity (lactic acidosis or hydrochloric acidosis) up to 70 units.

**M. PROTOZOA:**

Both ciliates and flagellates are present in rumen. But only ciliates are of physiological importance in virtue of their numbers and mass. The majority of ruminal protozoa belong to the family Obbryoscoleidae. Their numbers and size distribution provide information on protozoal activity within the forestomach.

Place a drop of rumen fluid on a slide and cover with a cover glass and examine under lower power (80 or 100 magnification).

**Assess the following Characters:**

1. **Density:**
   - ++++ Vigorous
   - +++ Abundant
   - ++ Moderate
   - + Few
   - — None
   - More than 30 Protozoa/low power field (100 X)
   - 10-30 Protozoa/L P F
   - 1-10 Protozoa/L P F

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Page 122
More: the density of protozoa, more active is the rumen fluid.

Simple inactivity of flora and fauna

Rumen decomposition/alkalosis

Acute rumen acidosis

2. Proportion of large, medium and small infusoria:
   - Large and medium ciliates in large numbers → Active rumen fluid
   - Only small ciliates → Forestomach indigestion
   - Total absence of ciliates → Entire microflora dead

3. Proportion of dead to live protozoa:
   - Rumen acidosis (pH less than 5) and severe digestive disorders like
   - Moderate digestive disorder → Proportion of dead to live increase

4. Iodophilic activity:
   - Add a drop of Lugol’s Iodine to 2-3 drops of rumen fluid on a glass slide and place a cover slip on it and examine under the microscope. Iodophilic activity of protozoa can be recognised by black colouration of starch contents in the protozoa. It can be graded as 0, +, ++, +++ depending on quantity of starch contained. The quantum of starch content of protozoa reflect the degree of digestive disease.

Total protozoal count

5 ml of strained rumen liquor (SRL) is diluted to 20 ml with 10% formal saline.

From this 10 ml of the mixed rumen liquor is taken and 10 drops of 2% Eosin is added to colour the protozoa. The diluted rumen liquor is charged in a Haemocytometer with Neubauer ruling. 18 sq. mm is counted and its average is multiplied by 50,000. The result is expressed as total counts per ml (n X 105).

Normal counts:

- Mixed ration: 1 X 105/ml.
- Concentrate ration: 1 X 106/ml.
- In disease: Decreased or Nil
- Bloat: Decreased total count, disappearance of Holotrichs
- Acidosis: Nil; if present, only few Entodinia sp

N. BACTERIA

An air dried smear of rumen fluid is stained by Gram’s method and observed under the microscope.

Interpretation

Basic criteria for interpretation are:

1) Presence or absence of morphologically distinguishable bacterial species characteristic of a normal rumen flora the so called leading bacteria
2) Multiplicity or uniformity of forms.
3) The ratio of gram positive to gram negative bacteria.
4) Comparison of the smear from the sick animal with that of the healthy animal receiving the same ration.

**Normal:** In the normal pH of rumen fluid - Gram negative bacteria is dominant.

**Rumen lactacidosis**
- Proliferation of gram positive cocci, and rods at the expense of gram negative bacteria.

**Hydrochloric acidosis**
- Gram positive less than Gram negative bacteria.

**Total bacterial counts**
- Add 5 ml of strained rumen liquor (SRL) and dilute to 25 ml with 10% forma saline. Take 1 ml of this fluid and is again diluted 100 times with 0.85% saline 0.01ml of this sample is spread over an area of one square centimeter making use of the guide plates. After the smear has dried, it is stained with Newman's stain. At least 30 fields should be counted, each smear, and the average count is to be multiplied by the dilution factor and in microscopic field. The result is expressed as total counts per ml (X x 108).

**Significance:** Normal 107 to 1012/ml.

**DIAGNOSIS OF TOXICITY BY RUMEN FLUID EXAMINATION**

**NITRITE/NITRATE TOXICITY**

**Modified diphenylamine test**
- A drop of the test solution (0.5 g of diphenylamine, 20 ml distilled water and concentrated sulphuric acid sufficient to make 100 ml) is mixed with a drop of rumen fluid. An intense blue colour within 10 seconds indicates a concentration of greater than 1% nitrate which is more than the permissible level.

**HYDROCYANIC ACID TOXICITY**
- Reagent papers are prepared by mixing 0.5 g of picric acid, and 5 g sodium carbonate in 100 ml water. Filter papers are dipped in the reagent and allowed to dry in a dark place. A drop of rumen fluid is placed on the test paper. A red discoloration is a positive reaction. The test is designed only to detect free hydrocyanic acid. It may not be positive when cyanides are present in different forms.