

Ruminal fluid examination

4/22/2020

Rumen fluid analysis

1

Introduction

- Microbes found in the reticulo-rumen include bacteria, protozoa and fungi.
- Bacteria and protozoa, are the predominant microbes and by mass they account for 80-90% of total microbial matter in the rumen.
- They are categorized into several functional groups, such as fibrolytic, amylolytic and proteolytic types.
- They are engaged in digestion of structural and non-structural carbohydrates, and proteins.

4/22/2020

Rumen fluid analysis

2

Introduction.....

- Protozoa (40-60% of microbial mass) derive their nutrients through
 - Phagocytosis of other microbes, and
 - Degrading and digest carbohydrates especially (starch and cellulose) and protein.
- Ruminal fungi make up only 5-10% of microbes.
- Despite their low numbers, they hydrolyse ester linkages between lignin, hemicellulose and cellulose that help break down ingesta particles.

4/22/2020

Rumen fluid analysis

3

INDICATIONS

- To establish an accurate diagnosis the rumen disease.
- To collect for therapeutic transfusion

4/22/2020

Rumen fluid analysis

4

Rumen fluid collection

- ✓ By oral/nasal passage of stomach tube.
- ✓ By needle puncture.
- ✓ From slaughtered animal
- Oral/ nasal tube collection is preferred to avoid risk of peritoneal contamination

4/22/2020

Rumen fluid analysis

5

General remarks

- The sample should be evaluated soon after collection to minimize the effects of cooling and air exposure on protozoal activity and pH.
- Estimation of biochemical characters can be delayed to 9 hours at room temperature and up to 24 hrs on a refrigerated sample.

4/22/2020

Rumen fluid analysis

6

Examination of Ruminant Fluid

Physical characters

- Color
- Consistency
- Odor
- Sedimentation activity test

Chemical characters

- pH
- Cellulose digestion test
- Glucose fermentation test
- Nitrate reduction test
- Rumen fluid chloride

Microscopical exam.

- Quantitative exam.
- Qualitative exam.

4/22/2020

Rumen fluid analysis

7

Physical characters

-Color

- Normal :
- Olive to brownish green (hay ration)
 - Deeper green color (green ration)
 - Yellowish brown color (grain or silage ration)
- Abnormal:
- Milky grey (grain overfeeding)
 - Darker greenish (ruminal stasis/decomposition)
 - Grey with clots of milk (calves with abomasal reflux)

4/22/2020

Rumen fluid analysis

8

Physical characters

-Consistency

Normal: Slightly viscous

Abnormal: Watery (Inactive bacteria or protozoa)
Excess frothy (Frothy bloat/ vagus indigestion)

-Odor

Normal: Aromatic odor

Abnormal: Ammonia smell (Urea poisoning)
Moldy rotting (protein putrefaction)
Sour odor (excess lactic acid/grain overfeeding)

4/22/2020

Rumen fluid analysis

9

Sedimentation Technique

- It provides rapid evaluation about protozoa activity
- Must be done soon after collection

Procedure

- Put a sample of rumen fluid in a test tube and let to stand.
- Measure the time needed for completion of sedimentation of fine particles and floatation of coarse solid particles
- Normal time is 4-8 minutes.

4/22/2020

Rumen fluid analysis

10

Sedimentation...

Abnormal time:

- Prolonged time indicate protozoa inactivity
- Very rapid sedimentation with no floatation occurs in :-
 - ✓Rumen acidosis.
 - ✓Prolonged anorexia.
 - ✓Inactive microflora from indigestible roughages.
- No appreciable sedimentation or floatation :-
 - ✓Frothy bloat.
 - ✓Some cases of vagal indigestion.

4/22/2020

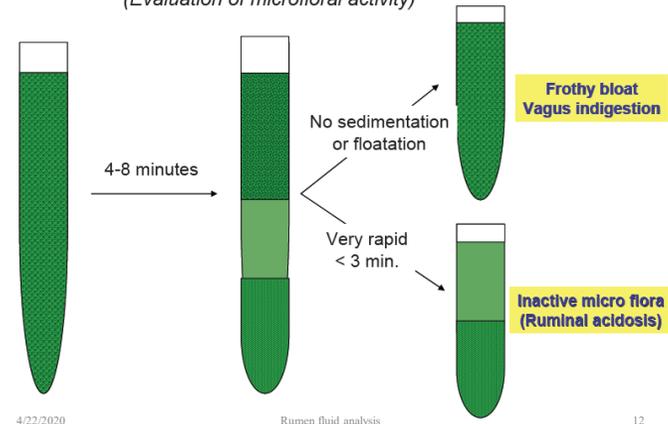
Rumen fluid analysis

11

Physical characters

-Sedimentation activity test

(Evaluation of microfloral activity)



4/22/2020

Rumen fluid analysis

12

Chemical Examination

pH

- It is measured by pH papers indicators or pH meters.
- It must be measured immediately after sampling.
- Normal pH differ according to type of food eaten
 - It ranges between 6–7 in animals feed on forage
 - Lower to 5.5 - 6.5 in animals mostly fed grain.

4/22/2020

Rumen fluid analysis

13

Chemical Examination

Elevated pH (Rumen Alkalosis)

- ✓ Simple indigestion
- ✓ Urea indigestion
- ✓ Putrefaction of rumen ingesta
- ✓ Feeding on indigestible roughages

Lowered pH (Rumen Acidosis)

- ✓ Readily digestible CH₂O
- ✓ Chronic ruminal acidosis
- ✓ Abomasal reflex from abomasal disease, Vagal indigestion and intestinal obstruction

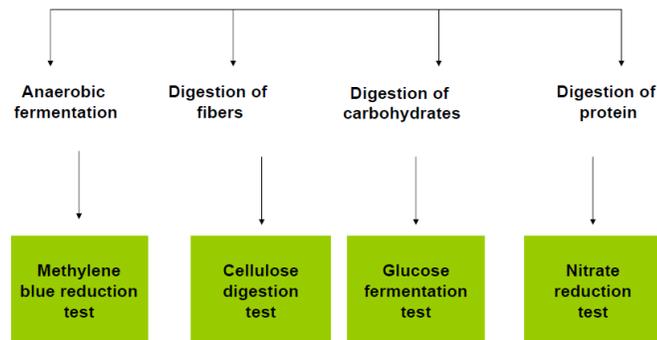
4/22/2020

Rumen fluid analysis

14

Chemical Examination

Ruminal flora and fauna



4/22/2020

Rumen fluid analysis

15

Methylene blue reduction Test

- It reflects the anaerobic fermentation done by respected bacterial population.
- Procedure:
 - Mix 12 ml of rumen fluid with 1 ml of 0.03% Methylene blue in a test tube
 - Let it to stand at room temp.
 - Measure the time needed the color change
 - Normal rumen fluid decolorize with 3 min

4/22/2020

Rumen fluid analysis

16

Methylene blue reduction Test...

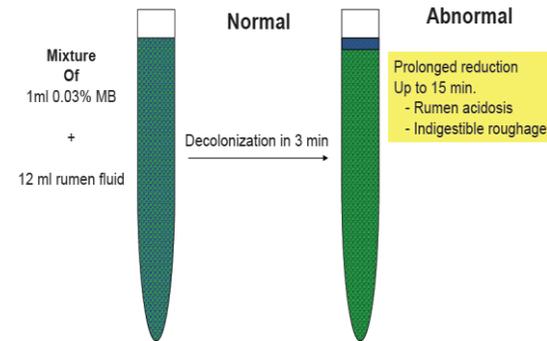
- Normal rumen fluid from cattle fed on hay and green needs 3 min to decolorize with leaving a narrow ring of blue color at the top
- Abnormal reduction may rise up to 15 min and indicates:
 - ✓ Indigestible roughage
 - ✓ Anorexia of several days
 - ✓ Rumen Acidosis

4/22/2020

Rumen fluid analysis

17

- Methylene blue reduction test



4/22/2020

Rumen fluid analysis

18

Cellulose Digestion Test

- It is important to evaluate the action of cellulytic bacteria

Procedure

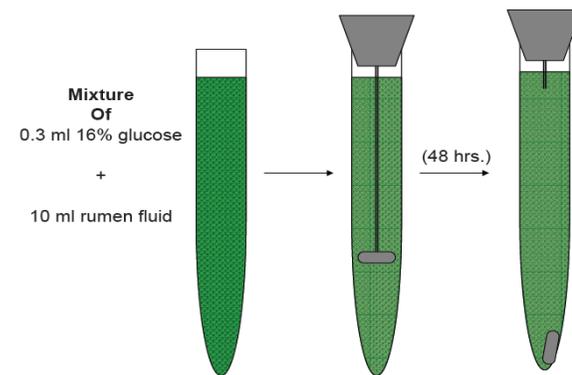
- Mix 10 ml rumen fluid with 0.3 ml of 16 % glucose
- Immerse a thread of pure cellulose by tie the lower end to a glass bead.
- Incubate the tube at 39 °C for 48-72 hrs
- Observe the bead to be dropped freely at the bottom of the tube.
- Interpretation:
 - A fully active rumen fluid will digest the cellulose within 48-56 hours

4/22/2020

Rumen fluid analysis

19

- Cellulose digestion test



4/22/2020

Rumen fluid analysis

20

Glucose Fermentation Test

- The test measure the ability of respected ruminal flora to ferment glucose

Procedure:

- 0.5 ml of 16 % glucose is added to 10 ml of rumen fluid
- Place the mixture in a fermentation saccharometer at 39 °C
- Read the result after 30 and 60 min for the formation of gas

4/22/2020

Rumen fluid analysis

21

Glucose Fermentation Test

Interpretation

- The test measure indirectly the ability of flora to break down (ferment) glucose through measuring the volume of gas formed.
- The normal rate of gas formation is 1-2 ml per hour.
- If the microflora is inactive, little or no gas will be formed.
- In case of foamy bloat more gas will be formed with pronounced foaming.

4/22/2020

Rumen fluid analysis

22

Rumen fluid chloride

- The Chloride concentration test is often of interest in cases of possible pylorus obstruction/blockage.
- Because elevated levels of chloride indicate reflux of the abomasum.

Procedure

- Take a supernatant of a centrifuged sample
- Measured by chloride meter.
- Normal level is 30 mEq/l
- Elevated level :
 - ✓ Abomasal disease.
 - ✓ Abomasal reflux.
 - ✓ Obstruction of intestinal flow

4/22/2020

Rumen fluid analysis

23

Nitrate reduction test

- It provides an idea about the activity of microbes that degrade and synthesize nitrogen compounds.

Procedure:

- 10 ml of sieved rumen fluid is placed into each of three tubes.
- 0.2, 0.5, 0.7 ml of 0.025 % potassium nitrate solution is added to the three tubes.
- Keep the tubes in a water bath at 39 °C.

4/22/2020

Rumen fluid analysis

24

Nitrate reduction test

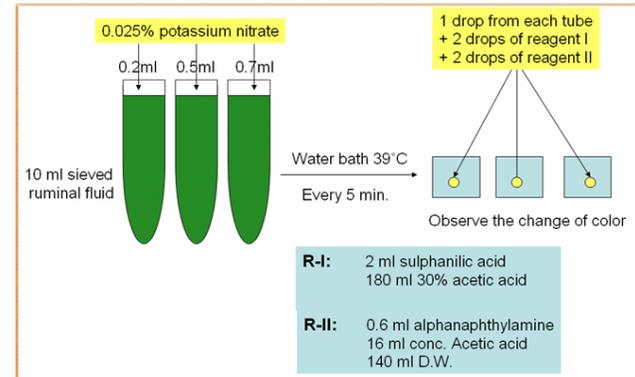
- Every five minute one drop from each tube is placed in small ceramic plate.
- To each drop add 2 drops of reagent 1
 - (2 ml of sulphanic acid in 180 ml of 30 % acetic acid)
- 2 drops of reagent 2
 - (0.6 ml alpha-naphthylamine + 16 ml conc. Acetic acid + 140 ml distilled water)
- Observe the color change to red

4/22/2020

Rumen fluid analysis

25

Nitrate reduction test....



4/22/2020

Rumen fluid analysis

26

Nitrate reduction test...

Interpretation

- Samples that contain microbes act on nitrates are colored red.
- Rumen fluid without the interest of microbes will not make color change with in 5-10 min in tube I and 20 min in tube II and 30 min in tube III.
- Reduction is more rapid when cattle are fed green fodder or have ruminal decomposition or bloat.
- Reduction is more slower when a deficient ration is fed or when the animal lacks appetite.

4/22/2020

Rumen fluid analysis

27

Microscopic examination

1. Qualitative method (Motility of ruminal protozoa)

Procedures

- Prepare a fresh film.
- Examine by low power.
- The activity of the fauna is judged as follow:

Motility	Activity
-Highly motile and very crowded	+++
-Motile and crowded	++
-Sluggish motility and low numbers	+
-No or sporadic alive fauna	0

4/22/2020

Rumen fluid analysis

28

Microscopic examination

2. Quantitative evaluation of rumen protozoa.

Technique

- Strain rumen fluid sample.
- Dilute 1 ml of strained sample with 15 ml saline solution and 5 ml lugol's iodine solution and shake gently.
- Spread 0.1 ml of the mixture on glass slide in an area under cover glass of 22 X 50 mm.
- Counting is carried out using low power (X 10). The field area of the lens is one square millimeter.

4/22/2020

Rumen fluid analysis

29

Microscopic examination

- Count 30 fields in the slide. The average count in 30 fields represents the protozoal count in one square millimeter area of the field.
- Multiply the average by 1100 to have protozoal count in 0.1 ml of the diluted fluid which represents 0.02 ml of the original sample.
- Multiply the obtained figure by 50 to obtain total protozoal count per ml.

4/22/2020

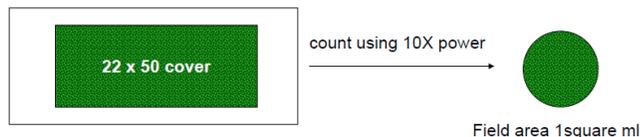
Rumen fluid analysis

30

Quantitative method

- Mix 1 ml of strained rumen fluid with 15 ml of saline and 5 ml of lugol's iodine.
- Spread 0.1 ml of the mixture on glass slide under

0.1 ml of the mixture



- Count in 30 fields.
- The average = protozoal count in 1square ml
- The average X 1100 (22x50) = protozoal count in 0.1 ml of diluted rumen fluid
- This number = protozoal count in 0.02 ml. of original sample
- protozoal count /ml = previous number x 50

4/22/2020

Rumen fluid analysis

31

Microscopical examination

Bacterial Identification

- Bacterial identification is generally accomplished through Gram Staining techniques
- Even if gram positive bacteria is observed, the fluid predominantly will be populated with gram negative bacteria.
- But in case of lactic acidosis, larger and more uniform population of gram positive bacteria may be observed.

4/22/2020

Rumen fluid analysis

32

Microscopical examination

Techniques

- Make filtration to the ruminal fluid
- Centrifuge to the filtrate
- Discard the supernatant and make a smear by taking the sediment
- Stain with gram stain and examined microscopically
- Under normal condition → more gram negative bacteria (red color)
- Abnormal condition → More gram positive bacteria (violent color)

4/22/2020

Rumen fluid analysis

33

Microscopical examination

Fungi

- Are present as yeasts (Filamentous organism)
- Such micro-organisms are sometimes present in exceptional numbers during rumen acidosis.

Procedure

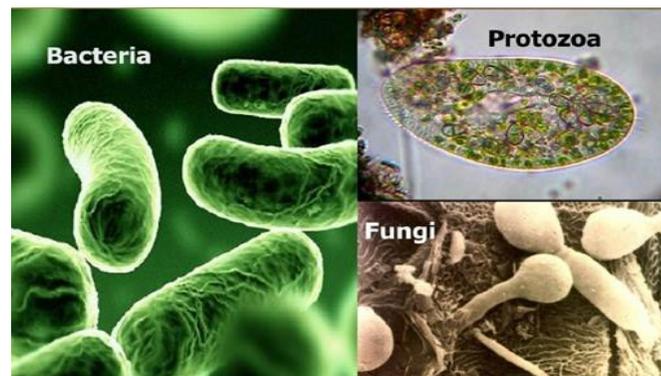
- Take a drop of sample from supernatant
- Stain with lugulo's iodine
- Examine under microscope with lower power

4/22/2020

Rumen fluid analysis

34

Microscopic image of rumen flora



4/22/2020

Rumen fluid analysis

35

Pancreatic Function Test

- Microscopic examination of feces for undigested food.
 - ✓ Presence of Fat
 - ✓ Presence of striated muscle
 - ✓ Presence of starch

4/22/2020

Rumen fluid analysis

36

Pancreatic Function Test

Presence of Fat

- Mix small portion of faeces with water or saline on a slide.
- Add equal amount of Sudan III (equal parts of 70% alcohol and acetone with an excess of Sudan III stain).
- Examine under low power objective, after placing a cover slip on the mixture.
- Fat will appear as orange or red globules, indicating a deficiency of pancreatic lipase.

4/22/2020

Rumen fluid analysis

37

Pancreatic Function Test

Presence of striated muscles

- Dilute faecal matter as above.
- Examine under microscope.
- Muscle fiber that appear as light yellow with cross striations is readily visible due to fiber remain undigested.
- It indicates lack of trypsin.
- The test has less clinical importance for ruminants.

4/22/2020

Rumen fluid analysis

38

Pancreatic Function Test

Presence of starch

- Make a direct faecal smear (as for Parasitological examination)
- Add one drop of alcoholic Sudan III solution and one drop of Iodine solution.
- Examine under microscope.
- Starch granules stain a blue black color.
- This indicates lack of amylase.

4/22/2020

Rumen fluid analysis

39



4/22/2020

Rumen fluid analysis

40