

## **Ruminal Fluid: A Review**

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### **Abstract**

*The analysis of rumen fluid is carried out to assess the function and activity of the ruminant fore stomach as well as the diagnosis of diseases of the fore stomach. This is a task for which the veterinary technician may be quite useful providing that they are familiar with ruminant anatomy as well as physiological considerations regarding handling and testing of collected fluids. The rumen microbial ecosystems coming to be recognized as a rich alternative source of genes for industrially useful enzymes. This review encompasses the various tests, several features and beneficial aspects of ruminal fluid.*

**Keywords:** *Ruminal fluid, Ruminant, Stomach, Enzymes, Microbes.*

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### **1. Introduction**

The ruminant fluid was identified as the potential source for biogas producing microorganisms such as hydrolytic, acidogens, acetogens, and methanogens. The analysis of ruminal fluid and its key examination are carried out, because it is an immense source of major beneficial organisms (Chuba, L, 2009). The observation of rumen fluid is done to analyse the activity of the ruminant fore stomach as well as the diagnosis of diseases of the fore stomach. This is a task for which the veterinary technician may be quite availed providing that they are familiar with ruminant anatomy as well as physiological considerations regarding handling and testing of collected fluids. Subacute ruminal acidosis is a common health and production problem in the cattle industries of the United States. It is a risk wherever cattle are fed diets rich in starch in order to achieve high rates of growth or milk production. For a variety of reasons, subacute ruminal acidosis has been a difficult diagnosis to make in the field. Foremost, the absence of specific diagnostic tests has made the diagnostician dependent on recognizing the syndrome of characteristic secondary clinical signs within the herd. A tentative diagnosis of subacute rumen acidosis is usually confirmed by the herd response to corrective nutrition. If the diagnosis proves to be correct, fault will be assigned to either the nutritionist who formulates the ration, the feed supplier who may mix and deliver feeds to the dairy or to the herd owner or manager who is responsible for the feed delivery system. Into this arena of vested interests, a veterinarian with modest or poor training in nutrition has to justify the tentative diagnosis based upon secondary signs of the problem. The situation is a potential point of stalemate or conflict in the resolution of herd problems, and begs for a more objective diagnostic approach (Radostits, O. M. et al., 1994).

It is important to have a working knowledge of the ruminant digestive tract when attempting sampling of rumen fluid. The ruminant digestive system is made up of four compartments: rumen, reticulum, omasum, and abomasum with each section being responsible for specific digestive functions. The 'fore stomach' contains the rumen, reticulum and omasum while the abomasum is regarded as the 'true stomach'.

Basic digestion within ruminants begins with the animal chewing up food that is subsequently mixed with bicarbonate/ phosphate enriched saliva. Once swallowed, the fore stomach begins to contract mixing even more buffers with the forage. At this point, the animal regurgitates their food and re-chews it. This is commonly known as 'chewing cud', an action that allows for more saliva to be mixed with foodstuff before being swallowed. The food then moves throughout the other three compartments for complete digestion.

The rumen, the first compartment to contribute to digestion is itself an ecosystem populated with a variety of unicellular organisms. These organisms are responsible for digestion of foodstuffs, utilization of resulting sugars and acids, synthesis of vitamins and production of waste products such as Methane. The rumen fluid when collected and analyzed allows the clinician to assess the number of functional anaerobes, assess the functional capability and monitor output of waste materials (Anderson, D., 2009).

## 2. Method of Collection

There are three methods of rumen fluid collection:

- a) Needle puncture of the rumen.
- b) 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.
- c) Manual method from slaughtered animal

### *Rumenocentesis Procedure*

Rumenocentesis, the collection of rumen fluid by percutaneous needle aspiration, has become a common diagnostic test in the United States. The technique has been described in detail (Bowen, R., 2009) but essentially involves inserting a needle into the ventral rumen and aspirating a sample of rumen fluid. Landmarks for the puncture site are the left side on a horizontal line level with the top of the patella about 15 to 20 cm posterior to the last rib. The site is clipped and prepared using a standard three scrub surgical preparation. Sedation or local anesthetics are not used. Rather, the cow is restrained in a stanchion or head-lock and one assistant elevates the tail of the cow while another assistant inserts a "nose leader" and pulls the cow's head to the right side. The clinician inserts the needle within a few seconds after the nose-leader is inserted. A disposable needle of 4" or 5" length is thrust through the skin, then into the rumen and fluid is collected with very slight aspiration. The needle will usually become obstructed by ingesta which is cleared by forcing a small volume of air or fluid back through the needle. When the needle becomes obstructed, it is important to avoid creating a negative pressure within the syringe as CO<sub>2</sub> will leave the fluid and increase the pH. Typically, 3 to 5 ml of rumen fluid can be collected with minimal difficulty. When sufficient volume has been obtained, the air is evacuated from the syringe and pH is measured immediately (Nordlund, K. V. et al., 1994).

*Rumen Fluid Sampling: Diagnosing Rumen Acidosis*

Ruminal acidosis can be diagnosed by collecting samples of rumen fluid by rumenocentesis and measuring the pH of the fluid (Nordlund, K. V. et al., 1994).

*Collection Protocol*

- a) Samples of rumen fluid should be collected from a minimum of 6 early lactation cows and 6 mid-lactation cows.
- b) The samples of rumen fluid should be collected 2 to 4 hours after concentrates have been fed in component fed diets or 4 to 8 hours after total mixed rations have been fed.
- c) Nordlund has developed a system for classifying the results of rumenocentesis. If 30% or more of the cows in either groups have ruminal pH's of less than 5.5, the group has a problem of ruminal acidosis. If more than 30% of the early lactation cows have ruminal acidosis, then there is a problem with adaptation of the cows to lactating cow rations. If more than 30% of the mid-lactation cows have ruminal acidosis, then there is a ration formulation or feeding problem.

*Collection Technique*

- a) Collection site is from the ventral sac of the rumen which should be identifiable 15-20cm caudoventral to the costochondral junction of the last rib.
- b) The site is clipped and surgically prepped.
- c) The cow should be lightly sedated (20-25 mg Xylazine IV for adult cow) and preferably hobbled.
- d) A 16 ga 5 in stainless steel needle is used. Insertion through the skin is the most painful to the cow. When the cow calms down, the needle is inserted to the hub and rumen fluid aspirated with a 10-20 ml syringe.
- e) 3-8 ml of rumen fluid is sufficient. The pH should be measured immediately using pH paper or pH meter. We prefer the field ready, compact pH meters but narrow range papers that span the pH range of 4.0-7.0 showing gradients of 0.20-0.30 units are acceptable.

**3. Ruminal Fluid Examination: Relation of Microbes in Rumen**

Microbes in the reticulorumen include bacteria, protozoa, and 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. Ruminal ecosystem is an enriched source of various microbial floras. Majorly the bacterial flora contains the category of beneficial and energy efficient microorganisms such as hydrolysis, acidogenesis, acetogenic, methanogenesis; *Bacillus subtilis*, *Bacillus clausii*, *Bacillus flexii*, *Bacillus cereus*, *Clostridium sp.*, *Methanobacterium sp.* The presence of potential microbes produces several beneficial enzymes such as lipase, cellulase, carboxy methyl cellulase, gelatinase, protease, amylase etc.

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 digest particles.

- a) It is often essential to establish an accurate diagnosis of diseases of the rumen.
- b) It is also essential when rumen fluid is collected for therapeutic transfaunation.

#### *Examination of Ruminal Fluid*

- a) 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.
- b) Estimation of biochemical characters can be delayed to 9 hours in room temperature sample and up to 24 hrs on a refrigerated sample.
- c) Transportation of rumen fluid for long distances must be done through double Jacket container (Coleman).

#### *Time of Sample Collection relative to Feeding*

Samples should be collected at a time when rumen pH is likely to be near the lowest point of the day. If the ration is fed as separate components, rumenocentesis should be performed between 2 to 4 hours after the cow is offered the primary concentrate meal of the day. If the ration is fed as a total mixed ration (TMR), the samples should be collected at 4 to 8 hours after the cows get access to the fresh ration.

#### *pH Determination*

The use of a pH meter is recommended for the measurement. In field use, the meter is calibrated using standard solutions of pH 4.0 and 7.0, read the samples, and then checks accuracy of readings using the standard solutions again.

The alternative of pH indicator paper is problematic in that the gradations on the narrowest papers we can find are 0.3 pH units. Frequently, the colour cannot be matched to a single reference value. The combination of greenish rumen fluid and poor lighting in many barns present additional practical problems for pH papers.

While there are advantages in communication of results if the readings are done cow side, the samples can be collected and held cold for pH analysis later. In a particular research and review analysis, pH of 18 samples on farm, kept the remainder on ice in capped plastic syringes from which air was expressed, and retested in 7 hours. The mean change in pH was less than 0.05 pH units and did not change the diagnostic classification of any animal or group. This limited analysis suggests that the samples, if kept cold, can be held for pH determination in a laboratory later that day.

#### *Interpretation of Results*

Based upon literature reviews of rumen fiber digestion and the clinical impressions from using the rumenocentesis test in investigations of acidotic herds, it is recommended a pH of 5.5 as the cut-point between normal and abnormal (Nordlund, K. V. et al., 1995). Since that time, prospective studies have identified pH of 5.5 as the best cut-point to distinguish normal and fiber-deficient rations (Garrett, E. F. et al., 1999).

The guideline is that if 30% of 10 or more sampled cows are below 5.5, the group is classified as experiencing ruminal acidosis. It is important to avoid making a herd diagnosis based upon a few samples. We recommend that 10 or more cows should be sampled from any group in which acidosis is suspected. In a feeding trial of with a normal and a fiber deficient ration, we found a prevalence of 8% of the rumen samples below pH 5.5 in the “normal” high production ration and a prevalence of 40% below 5.5 in the “acidosis” ration. With these distributions of pH values, the interpretive guidelines offered above will be correct more than 95% of the time (Garrett, E. F. et al., 1999).

#### 4. Conclusion

Ruminal fluid from various ruminants, are being tested and examined with several parameters. Some of the criteria are discussed. There is ample potentiality for the microbes vested in the ruminal fluid. Thus, the biotechnological framework is in place to achieve and explore substantial improvements in animal production through various efficient enzymes producing microbial species. There is a major scope of research in ruminal ecosystem.

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