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Assessment of the Rumen Fluid of a Bovine Patient

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Review Article

Assessment of the rumen fluid is an ancillary examination technique used in the clinical examination both of the individual bovine patient and at population level. The specific function and structure of the rumen makes ruminants different from other animals. Therefore, it logical to assess the rumen in detail when confronted with a bovine patient. Assessment of the rumen fluid is essential to assist with the diagnosis of disorders of the rumeno-reticulum (e.g. indigestion, abomasal reflux, traumatic reticuloperitonitis), fermentation (e.g. subacute rumen acidosis (SARA)) and disruption of the passage of ingesta into the intestines (e.g. pyloric obstruction). Additionally, rumen samples are required to check the rumen health at an individual (e.g. selection of a suitable candidate as a donor for rumen transfaunation) or population level, particularly on enterprises that feed a high-energy, easy digestible diet (e.g. SARA). For this purpose, 12 individuals are usually selected randomly from each group on the farm and rumen samples collected for analysis. The volume of rumen fluid required for the above indications above is usually small (3-300mL). Collection of rumen samples is also required for rumen transfaunation. For rumen transfaunation, moderate quantities of rumen fluid are collected from donor cows using specialised equipment (e.g. Dirksen, Geishner or Sorensen and Shambaye probe), or from freshly slaughtered or rumen canniculated patients.

For consistent, repeatable and comparable results the methodology of rumen sampling should be standardised. To obtain the most representative sample, collection should be carried out at the nadir of the pH. The timing of collection depends on the type of diet. Cattle fed total mixed rations (TMR) should be sampled 4-8 hours after they have gained access to the fresh ration. Cattle fed on grass should be sampled 3-5 hours after gaining access to a fresh break of pasture. Cattle fed partially mixed rations (PMR) or separate components should be sampled 2-4 hours after the primary concentrate meal of the day (e.g. after milking when concentrate is offered at the milking parlour).

Technique of Rumen Fluid Sample Collection

A rumen fluid sample can be collected by passing a rumen tube (oro-ruminal or less commonly naso-ruminal) of variable diameter (with or without pump attached), via rumenocentesis or through a rumen cannula.

Passage of a Rumentube

Rumen fluid is most commonly obtained from cattle with a developed rumen (usually older than 3-4 weeks of age) via passing of a rumentube. To facilitate the collection of rumen fluid a suction pump may be attached to the oral (free) end of the tube.

Technique for Passing a Rumen Tube

The placement of a tube can be aided by a wooden gag with a hole through the centre or any other type of gag (e.g. Drinkwater gag). However, any large-bore tube covered with a metal spiral made specifically for oro-ruminal tubing that does not require the use of a gag and can be introduced directly into the oral cavity. Some tubes have a heavy head that ensures the tube sinks into the fluid content of the ventral ruminal sac (e.g. Geishauser oro-ruminal tube). The tube should be at least 2.5m long and rigid enough to pass through the rumen mat and enter into the fluid portion of the ventral ruminal sac. To prevent frequent blockage, the internal diameter of the tube should be at least 1cm. The tube should be checked for sharp projections before use. Access to the rumen usually requires at least 2 metres of tube to be introduced into a Holstein patient of 500-600kg body weight.

Aspiration can be carried out by suction by mouth or assisted by a pump. Suction by mouth may be sufficient for an individual patient. Pump-assisted suction is necessary for sampling of a population. (NOTE: Suction of rumen fluid by mouth is unpleasant for the practitioner and carries a risk of zoonotic disease transfer.)

Rumen fluid collected via rumen tube is often mixed with bicarbonate-rich saliva. This is a result of the tube coming into contact with saliva as it is being passed through the oral cavity, pharynx and oesophagus. The first 100mL of the collected sample are usually 10-25% saliva. Therefore, to prevent misinterpretation of rumen fluid composition and rumen health, in particular rumen pH, the minimum volume of the collected sample should be at least 200mL. Alternatively, discarding the first 50-100mL of the collected rumen fluid sample significantly reduces the effect of contamination with saliva. Another

alternative is to cover the tip of the tube with a wrap, finger of an examination glove or bag, and blow on it, forcing air in when the tube is in place in the rumen to remove the cover and saliva from inside the tube.

Passage of Naso-Ruminal Tube

The sample obtained by passage of a naso-ruminal tube is less contaminated with saliva than by passing an oro-ruminal tube as it does not pass through the oral cavity. The pH of a sample obtained via naso-ruminal tubing is usually lower than that from oro-ruminal tubing and slightly higher (0.1-0.2 point) than samples obtained via rumenocentesis. Therefore, the same preventive strategies as for oro-ruminal tubing (discarding the first 50-100mL of the collected fluid or collection of a minimum of 200mL) should be implemented when obtaining rumen samples via naso-ruminal tube.

Note: To prevent damage to the upper airways, the procedure of naso-ruminant tubing requires use of a soft tube that rarely reaches into the fluid content of the ventral sac of the rumen.

Rumenocentesis

Rumenocentesis or ruminal paracentesis is a technique involving percutaneous rumen puncture and aspiration of rumen fluid from the ventral ruminal sac. Rumenocentesis has the advantage overpassing a rumen tube to collect rumen fluid, due to the possibility of contamination with saliva when rumen tubing. Therefore, rumenocentesis is the preferred method of collection when assessing rumen pH. However the procedure is surgically invasive, and some clients are reluctant to implement it on their enterprise. Approximately 20% of sampled patients show decreased appetite and signs indicative of pain lasting up to 3-4 days following rumenocentesis. Additionally, 1-5% of sampled patients develop long-lasting complications including haematomas, abscessation and localised peritonitis. Furthermore, in a small portion of patients in late gestation the needle may penetrate the uterus, and in patients soon after calving the abomasum (e.g. early or asymptomatic left displaced abomasum). To avoid puncturing the uterus/abomasum a quick ultrasound examination of the area before rumenocentesis can confirm the position of the rumen. Finally, as with any other injection site, bleeding may occur by accidental penetration of a blood vessel and the rumen fluid sample may become contaminated leading to an inaccurate pH result.

The landmark for rumenocentesis for insertion of the needle is the intersection of a horizontal line from the point of the patella and a vertical line 15-20cm caudal to the last rib on the left side. Alternatively, rumenocentesis can be carried out over the left paramedian, just next to the umbilicus. This site is more risky for the practitioner due to the higher likelihood of being kicked.

The patient should be properly restrained for rumenocentesis. As the location to be sampled is within the striking area of the rear limb, it is advisable to hobble the rear limbs together or secure the near rear limb. Alternatively or in addition, a 'tail jack' by an assistant may be a useful restraint. Mild sedation (e.g. xylazine 0.04mg/kg BW administered IV) may also be required when sampling. At the same time, the head of the patient should be restrained by pulling it to the right, preferably using nose grips. Despite this, some patients are very reluctant to undergo the procedure and thrash around. The nature of rumen fluid makes collection of the sample under sterile conditionspractically impossible. However, the practitioner should still adhere to aseptic principles. A 5-10x5-10cm area should be clipped and surgically scrubbed. Local anaesthetic (i.e. lignocaine) should be infiltrated subcutaneously and intramuscularly in the sampling area (5-10mL is often sufficient).

The recommended needle size is 1.2-2.1mmx8-12cm (the most commonly recommended size is 1.6 mmx10cm). The needle should be attached to a 10 to 20mL syringe containing 3-10mL of air. An eccentric syringe is preferred. The needle is first introduced subcutaneously. This is the point at which the patient tends to show highest resistance. Thereafter, the needle is attached to the syringe it should be inserted firmly by a single thrust into the ventral sac of the rumen perpendicular to the skin. For puncturing the rumen to prevent contamination of the area the syringe should always be attached to the needle. Rumen fluid should be aspirated by applying very slight suction on the syringe. Some authors state that excessive suction on the sampling syringe can cause CO2 to leave the fluid and may alter the pH reading. The ventral sac of the rumen contains a mixture of fluid and particulate matter. Therefore the needle often becomes blocked. When the needle becomes blocked, aspiration should be stopped, the needle repositioned without being withdrawn, and 2-5mL of air forced back through the needle to clear the needle. After this aspiration of rumen fluid may be resumed. Use of an eccentric syringe makes the procedure of forcing air back easier. By holding the syringe with the eccentric tip uppermost, most of the collected fluid can be retained in the syringe whilst forcing air back through the needle. After collection of the sample the needle should be withdrawn rapidly without pulling on the plunger.

Up to 5mL of rumen fluid can be collected with minimal difficulty. The maximum recommended volume to be aspirated is 15-20mL. Where larger volumes are required alternative collection techniques (e.g. tubing) should be considered.

Rumen Cannula

The rumen cannula is surgically placed in the rumen. Once in place, it is the easiest way to collect a representative rumen content sample. However, it is not practical for collection of rumen content/fluid on many commercial dairy farms. Some farms have several cannulated cattle as donors for transfaunation however, this is not a common occurrence. Further discussion on this method of collection is beyond the scope of this writing.

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Assessment of the Rumen Fluid Sample

Examination of rumen fluid should establish the colour, odour, consistency, pH, sedimentation, rumen microbial population (rumen protozoa and rumen bacteria) and rumen chloride concentration. Other tests include cellulose digestion, glucose fermentation, nitrate reduction, rumen osmolarity, etc. However, these require specialised equipment, prolonged times to complete or yield little additional practical information. They are not discussed in this writing.

The rumen sample should be assessed as soon as possible after collection. Rumen samples kept for longer than 9 hours in air-tight containers at room temperature or refrigerated for longer than 24 hours should not be assessed. Delayed assessment should have only minimal changes to physical assessment. Delayed assessment of rumen fluid has the most significant effect on rumen pH. Additionally, cooling and exposure to air may significantly change the findings related to rumen protozoa.

Rumen Fluid Colour and Odour

The 'normal' colour of rumen fluid is olive-green to greenish-brown with a sweet and fermentative smell (often described as aromatic and non-repellent). This partially resembles the composition of the diet. Cattle fed on maize silage or straw have yellowish to brown coloured rumen fluid. Cattle on pasture have green coloured rumen fluid. Cattle fed on high concentrate rations often have olive-brown rumen fluid.

Abnormalities in Rumen Fluid Colour and Odour

Rumen fluid from a patient with lactic acidosis usually has a milky-grey colour and characteristic rancid/acidic smell. The smell of abomasal contents is indicative of pyloric outflow obstruction and/or backflow of abomasal content. Blackish-green colour with a foul, foetid and/or mouldy smell is usually seen in cases of putrefaction of rumen ingesta or due to prolonged ruminal stasis (e.g. vagal indigestion). A stale and indifferent odour is usually indicative of inactive rumen. A putrid odour detected by the rumen tube or in the rumen sample of young calves is often indicative of putrefactive indigestion (e.g. due to abomasal reflux or milk escaping the oesophageal groove deposited in large quantities in the developing rumen).

The colour of the rumen fluid may also change due to diet (e.g. feeding beetroot results in reddish tinge) or iatrogenically (e.g. administration of activated charcoal results in black colouration or bleeding due to rumenocentesis results in reddish discolouration).

Technique for Assessment of the Rumen Fluid Colour and Odour

The colour and odour of rumen fluid are assessed by visual inspection and smell. The colour is best assessed in a transparent plastic or glass tube of a smaller diameter (<1.5cm). The assessment should ignore the colour of the admixtures (e.g. large feed particles). Therefore, a sample rich on large particles is best sieved (e.g. through a gauze swab) before rumen fluid colour is assessed. The odour can be assessed on samples of any age. For improved detection of the faint odours, the sample should be closed in an air-tight container for a minimum of five minutes and then opened to assess the odour.

Rumen Fluid Consistency

The consistency of rumen fluid in 'normal', healthy cattle is slightly viscous.

Abnormalities in rumen fluid consistency

The consistency of rumen fluid changes from slightly viscous in normal, healthy cattle to watery in cases of rumen dysfunction and inactivity. A very watery sample, with little particulate matter is indicative of starvation. Such samples usually have reduced microbial activity (e.g. low protozoa, increased sedimentation time and prolonged methylene blue reduction test). Samples of rumen fluid which contain medium to large bubbles that do not coalesce are highly indicative of frothy bloat.Sample of rumen fluid with pasty consistency that contain a large number of small bubbles are indicative of vagus indigestion.

Technique of assessing rumen fluid consistency

Rumen fluid consistency is assessed by slowly turning a glass or plastic tube half-filled with rumen fluid 45-60° left and right from an upright position. If this motion is too fast foam may form on the top of the sample. The assessment of the rumen fluid consistency should also assess for evidence of bubbles.

An excessively viscous sample is probably predominantly composed of saliva. It is unlikely that such a sample will give representative results during the rumen fluid health assessment, particularly regarding the rumen fluid pH. Therefore, when a rumen fluid sample obtained at the first sampling attempt is highly viscous repeated sampling is indicated prior to assessing the sample.

Rumen Fluid pH

The physiological pH of the rumen fluid and contents varies significantly depending on the type of diet, time between feeding and sampling, and the portion of the rumen where the sample has been obtained from. The 'normal' pH of the rumen fluid, collected by rumenocentesis in cattle on an exclusively pasture diet is 6.0 to 7.2. A rumen pH of 5.0 to 6.0 may be normal in cattle on a high, easy-digestible carbohydrate diet (e.g. high concentrate or lush pasture) for the first 6 to 24 hours. A rumen pH of 6.0 to 6.8 is 'normal' for cattle on a diet rich in crude fibre.

Abnormalities of the rumen fluid pH

An acidic pH of the rumen fluid is associated with over-feeding of rapidly fermented carbohydrates (e.g. cereal meal, sugar beet, molasses, etc.). A low rumen pH (pH of 4.0-5.0) confirms a diagnosis of rumen acidosis.However, a long-lasting pH of less than 5.5 is assumed to indicate subacute ruminal acidosis (SARA). The diagnosis of SARA is not straightforward. It should be diagnosed at a herd level, and not based on the pH

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measurement of a single patient. Additionally, other evidence of SARA must be present in the population of interest. The diagnosis of SARA is usually confirmed when >3/12 tested patients have a rumen pH <5.5. To complicate things further, some authors mention >4/12 patients with a rumen pH of <6.0 on rumen fluid samples collected by rumenocentesis. The diagnosis should also take into account signs indicative of SARA including variable faeces quality (often sticky), reduced and variable feed intake, variable and reduced milk fat content, decreased milk fat/milk protein ratio, sole haemorrhaging, coriosis, poor body condition, perineal faecal soiling, increased risk of premature culling, high rates of abomasal displacement, reproductive failure and treatment of metritis and mastitis. On some enterprises, there is also increased prevalence of caudal vena cava thromboembolism, evident by bilateral epistaxis and sudden death. NOTE: Low rumen pH may also result from reflux of abomasal content (e.g. abomasitis, anatomical or functional stenosis of the pylorus and bovine leucosis).

Rumen pH may increase and become alkaline (pH 7.0 to 8.0) after withdrawal of feed for 24 hours or longer. The pH is also alkaline when microflora is inactivated. A rumen pH of 8.0 to 10.0 is abnormal and can be detected in cattle with urea toxicity or protein putrefaction into the rumen. However, a high rumen pH (>8.0) may be detected in cattle where the rumen sample is mixed with a high volume of saliva. Rumen fluid collected through a rumen tube with a pH of 7.5-8.5 or even higher, with active protozoa, normal sedimentation and viscous appearance is indicative of contamination with high proportion of bicarbonate-rich saliva.

Technique for assessing rumen pH

Rumen fluid pH can be measured using narrow range pH indicator papers or, ideally, by a pH meter. A variety of pH meters can be used. For field situations a compact, hand-held pH meter that requires only a small volume of fluid with a calibration liquid is preferred. For accurate results, the practitioner must adhere strictly to the instructions provided by the manufacturer. The pH indicator papers are usually not sensitive enough to detect pH changes of 0.1 points. Furthermore, the colour change is often comparable to few values on the chart. Finally, the pH paper technique requires a good natural source of light, restricting the availability of the test.

Rumen fluid pH should be measured as soon as possible after collection of the sample (within minutes). Delays in measuring rumen pH may produce inaccurate results. Fermentation of the rumen fluid sample continues after collection. Additionally, CO2 escapes from the sample. These two factors may lead to a relatively rapid change in rumen pH of a sample kept at room temperature or in open containers. Alternatively, if measurement of rumen pH is postponed, the sample should be refrigerated in an air tight container. The pH should be measured in the next 4-10 hours. Prolonged storage (>12 hours) usually results in inaccurate pH readings.

The pH of the rumen samples collected by oro-ruminal or naso-ruminal tubing are usually 0.2-1.0 and 0.1-0.2 points respectively higher compared to samples collected by ruminocentesis. Therefore, rumen pH of samples collected by oro/naso-ruminal tubing is very specific but may lack sensitivity.

Rumen Fluid Sedimentation

Sedimentation of the rumen content is often a very rewarding ancillary technique that is highly underutilised in practice. In normal, healthy cattle the finer particles will sink to the bottom of a test tube in 5-10 minutes.

Abnormalities in rumen fluid sedimentation

With an abnormal rumen sample (e.g. lactic acidosis), the sedimentation rate is fast (often less than 2 minutes) with a delayed or absent secondary flotation. In cattle that have been starved for 24 hours or longer rumen sample may show very rapid sedimentation and secondary flotation. Rapid secondary floatation and abundant foam is indicative of rumen sample that has undergone decomposition (e.g. rumen putrefaction). In such fluid, the large particles do not float and remain in suspension for a long time.

Technique for assessing rumen sedimentation

The test should be carried out on a fresh sample (less than 2 hours old) in a plain test tube. For easier assessment the sample can be filtered through a gauze swab. Normally, fine particles rapidly settle at the bottom of the tube and coarser particles are carried upwards with the gas bubbles resulting from fermentation leading to formation of a foaming upper layer. In cattle with an active rumen, the sunken fine particles will later rise to the surface (secondary flotation). The assessment is based on the time required for the sedimentation of the fine particles and floatation of the coarser particles to occur. In some assessments, the presence and speed of the secondary floatation is also used.

Rumen Fluid Protozoa

The presence, diversity and activity of the rumen protozoa (rumen fauna) are indirect indicators of the health of the rumen. Rumen protozoa are highly reactive to changes in their environment. They may well provide the most sensitive measure of rumen health and function. 'Normal' rumen fluid should contain variety of species, sizes and forms of ≥40 protozoa per microscopic visual field. The protozoa should be active. 'Normal' rumen fluid should have >10 protozoa entering a single microscopic field over a period of 30 seconds. Ciliated and flagellated protozoa should be detected. Cattle on a high grain diet usually have predominantly flagellated protozoa. Cattle on a high roughage diet usually have predominantly ciliated protozoa. Three sizes of protozoa should be present: small, medium and large.

Protozoa possess a minimum level of energy reserves which are easily visible on staining with Lugol's iodine. 'Healthy'

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protozoa should be almost uniformly stained with the Lugol's iodine.

Abnormalities in rumen fluid protozoa

Low numbers of protozoa (<8 per field), inactive protozoa (<5 entering the field), loss of diversity or any combination of the above are indicative of a 'non-healthy' rumen environment (e.g. decreased motility or absence of live protozoa may be detected in cases of lactic acidosis).Decreased activity of rumen protozoa in cases of rumen acidosis can be detected even before the rumen pH has fallen below the critical level. In advanced rumen acidosis rumen protozoa can no longer be found. Protozoa with insufficient starch reserves have decreased numbers of starch granules when stained with Lugol's iodine.

Technique for assessing rumen fluid protozoa

Rumen protozoa are assessed by viewing a drop of fresh rumen fluid using the low power objective (40x magnification) on a warm microscopic side at about 30°C. Rumen microbial population can be assessed by counting the number of protozoa present per field and protozoa entering a single microscopic field in 30 seconds.

Estimation of the energy reserves can be made by mixing one drop of rumen fluid with the same volume of Lugol's iodine. Lugol's iodine stains the starch granules inside the protozoa. The assessment is made using low (40x) or medium (100x) power objective on 2-4 microscopic fields.

Rumen Fluid Bacteria

The rumen bacteria (rumen microflora) is comprised of a large number of species with a variety of morphological forms.

Decolourisation of methylene blue added to 'normal', healthy rumen fluid usually takes 2-6 minutes. Cattle fed on a high-grain diet with highly active microflora decolourise the mixture in less than a minute. A highly active microflora decolourises the methylene blue mixture within 3 minutes. Cattle fed on high-fibre diet have lower microbial activity.

Rumen fluid from 'normal', healthy cattle should have only a small percentage of Gram-positive bacteria. Cattle fed predominantly on roughage should have a high proportion of large bacterial forms. Cattle fed on mixed rations should have mixed-size bacterial forms. Cattle fed on high-grain diets should have relatively a uniform proportion of small bacterial forms.

Abnormalities in rumen fluid bacteria

Disappearance of the methylene blue discolouration which takeslonger than 10 minutes is indicative of poor microbial activity. Lack of active microflora is indicated when there is failure to decolourise the sample within 15-20 minutes. Such a finding indicates that rumen transfaunation is required.

Absence of Gram-negative bacteria in the rumen sample is an abnormal finding. A high proportion of Gram-positive bacteria (sometimes no Gram-negative bacteria at all) is indicative of lactic acidosis. Abnormalities in bacterial populations are also indicated when the morphology of bacteria does not represent the diet or when there is unusual uniformity (e.g. one or two forms and sizes predominate).

Technique for assessing rumen fluid bacteria

The presence of anaerobic bacteria can be confirmed using the methylene blue reduction test. It must be carried out shortly after collection. The test is invalid on rumen fluid sample with a pH of <5.5. It is carried out by mixing 0.5mL of 0.04% new methylene blue with 10mL rumen fluid at body temperature in a plain tube with the cap on. The time to decolourisation from blue to 'normal' is measured. For comparison, a tube filled with the original rumen fluid sample is held next to the test tube. For most accurate results, rumen fluid should be added to the methylene blue rather than adding the dye to the fluid.

A similar test can be carried out by mixing 0.5mL of 0.01% reassuring in 10mL of rumen fluid. Times taken to decolourise the mixture should be halved from the methylene blue test.

Carrying out a Gram-stain on an air-dried smear of rumen fluid is also a useful ancillary technique. To detect subtle differences in the microscopic assessment of a Gram-stained smear it is recommended to make a comparison with rumen fluid obtained from 'healthy' cattle fed on the same diet. Further tests including division in cellulolytic, proteolytic, methane-producing, starch or sugars fermenting and other groups of bacteria can be carried out but rarely have practical importance and are not discussed.

Rumen Fluid Chloride

Normal chloride concentration in the rumen fluid is <30mEq/L.

Abnormalities in rumen fluid chloride

Rumen chloride concentration of above 30mEq/L indicates reflux of abomasal content into the rumen (abomasal pH is 2.0 to 4.0). Another reason for high chloride content can be due to supplementation of the diet with salt. Abomasal reflux ('internal vomiting') may occur due to functional or anatomical stenosis of the pylorus, abomasitis, abomasal ulceration, obstruction of the flow of ingesta (e.g. bezoars, send, stones, and abomasal displacement) or vagus indigestion. Abomasal reflux may also occur in the digestive form of bovine leukosis and cellulitis of the adjacent mesenterium. Finally, abomasal reflux may also occur due to ileus of the cranial intestine (Table 1).

Technique for assessing rumen fluid chloride

For measurement of the chloride concentration, the rumen fluid sample should be centrifuged and the test should be carried out on the supernatant. This testing procedure is usually carried out at a laboratory. An estimate of the rumen chloride can be obtained by portable chloride meters, some of which are hand held and can be used in the field.

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In calves, renin is present in abomasal but not in rumen fluid. Therefore, a renin test will confirm the presence of abomasal reflux or milk accumulation in the rumen due to an oesophageal groove overflow. The test is carried out by adding 2mL of the tested rumen fluid to 2mL of fresh milk in a suitable container (i.e. sampling bottle). Coagulation of the milk confirms the presence of abomasal content in the rumen fluid.

Table 1: Common findings and their interpretation on assessment of a sample of the rumen fluid. Italicised text means abnormal finding or its interpretation.

Finding	Interpretation	Finding	Interpretation
Colour		**pH**	
Yellow/Brown	Corn silage/straw diet	6.0-7.0	'Normal'
Brown/Olive	Concentrate-based diet	5.0-5.6	High grain or lush pasture diet
Green	Pasture-based diet	5.0-5.5	Subacute rumen acidosis
Milky grey/Brown	Lactic acidosis	<5.2	Lactic acidosis
Light brown	Simple indigestion	7.5-8.5	Starvation/Simple indigestion
Dark brown/Black/ Darker greenish (non-pasture-based diet)	Putrefaction/Rumen blockage	>8.0-10.0	Urea poisoning
Grey (with clots of milk)	Abomasal reflux (calves)	>7.5	Putrefaction/Protein decomposition
Odour		**Bacteria**	
Aromatic	'Normal'	Gram negative >> Gram negative	'Normal' and Alkalosis
Acidic/Sour/Rancid	Lactic acidosis	Gram positive >> Gram negative	
Foetid	Rumen putrefaction	Gram negative > Gram positive	Clinical acidosis
Abomasal	Abomasal reflux		
Ammoniacal	Urea toxicity		Subacute rumen acidosis
Consistency		**Methylene blue reduction test**	
Slightly viscous	'Normal'	2-6 minutes	'Normal'
Excessively viscous	Contamination with saliva	<1 minute	Concentrate-based diet
Watery, very few particles	Starvation	>6 minutes	Lactic acidosis/Simple indigestion/ Starvation/Ration poor in structure/Indigestive fibre
Bubbles (large)			Microflora inadequate
Bubbles (small)	Bloat		Microflora has died/Vagus indigestion
Putrefying milk	Vagus indigestion	>10 minutes	
Clotted milk	Rumen drinkers (calves)	>15-20 minutes	
	Abomasal reflux (calves)		
Sedimentation			
5-10 minutes	'Normal'		
<2 minutes and retarded or absent secondary flotation	Acidosis/Simple indigestion/ Starvation		
Rapid secondary floatation			
>10 minutes			
>15 minutes	Rumen putrefaction		
	Rumen bloat		
	Vagus indigestion/Lactic acidosis		

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