Rumen fluid sampling via oral stomach tubing method

Wouter Muizelaar[1]
Paolo Bani[2]
Björn Kuhla[3]
Mogens Larsen[4]
Ilma Tapio[5]
David Yáñez-Ruiz[6]
Sanne van Gastelen[1]

[1]Wageningen University & Research, Wageningen, Niederlande
[2]Università Cattolica del Sacro Cuore, Piacenza, Italy
[3]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf,Germany
[4]Aarhus University, Tjele, Dänemark
[5]Natural Resources Institute Finland (Luke), Jokioinen, Finnland
[6]Estación Experimental del Zaidín (CSIC), Granada, Spain

Keywords: vacuum pump, RUMINATOR, FLORA, saliva, transfaunation, oesophagus

Introduction

There are different purposes for collecting rumen fluid, e.g. assessment of rumen fermentation characteristics and rumen microbiota, as well as *ex vivo* incubations for feed analysis. The rumen content is generally divided into three major phases; the liquid, the floating, and the gaseous phase, from ventral to the dorsal side of the rumen respectively. The liquid phase is often desired to be sampled and contains soluble fermentation products, saliva, as well as insoluble components such as sand, feed particles, and microbes. Due to the anatomical structures between the oesophagus and the rumen, the composition of the liquid phase differs between rumen compartments. Generally, the dorsal-cranial part of the rumen has higher saliva proportions, whereas the middle and caudal parts represent all components with less amounts of saliva [1], [2]. Therefore, the pH, the volatile fatty acid (VFA) composition, and the microbial composition differ between the rumen compartments [1], [2], [3].

Collecting rumen content through the fistula from rumen cannulated ruminants is considered the reference method for the collection of representative samples of rumen digesta, but access to surgically-modified animals is not universal and restricted to research facilities. Hence, a less invasive alternative has been developed: the oral stomach tubing (OST) technique. The OST technique allows rumen fluid to be obtained through the oesophagus. Execution of the OST technique is not without difficulties, such as resistance by the animal, consistent positioning of the OST sampling probe in the rumen, saliva contamination in the samples, and different protocols used worldwide. Also, individual animal characteristics have a large influence. For example, the position of the OST probe in the rumen is affected by the size of the animal, by the insertion depth of the OST tube and by the density of the fibre mat [3], [4], [5], [6]. A denser fibre mat in the rumen hinders the penetration of the OST sampling probe, causing the OST sampling probe to be more at the central to caudal and dorsal side of the rumen, rather than the ventral side of the rumen during sampling. These before-mentioned factors result in variation, particularly in the quality of the rumen fluid collected and the variables measured in the rumen fluid (e.g. microbiota), which justifies the need for standardization. This chapter, therefore, proposes guidelines based upon best practices by researchers in the field when performing the OST technique with intact ruminants and offers practical solutions to common problems. The aim is not to perform a literature review on how the OST technique affects certain parameters, but to provide guidelines to increase uniformity in the execution of the OST technique and to enhance the comparability and repeatability of research. Most of the work is based on adult cattle and some special remarks are given when working with young animals or small ruminants. The guidelines presented in this chapter can be used when the OST method is applied to sample rumen fluid from ruminants for the analysis of pH, VFA and other metabolites, or microbiota.

10.5680/mcpb008

Several devices are available for collecting rumen fluid samples via the OST technique. Here, we highlighted two types of devices which are most frequently used and explained their differences. There are multiple versions of these devices produced by different manufacturers. In general, scientific reports should mention at least the following characteristics when using any type of OST device for sample collection: manufacturer, product type, diameter and length of the sampling probe and tube.

A passive fluid collector works via valves opening as soon as the probe is fully submerged in the rumen fluid. An example of a passive fluid collector is the FLORA sampling device (Profs Products, Wittibreut, Germany) [7]. This type of device is designed for taking quick rumen fluid samples and minimizing the handling of the animal. The probe passes relatively easy through the oesophagus due to its small size. The animal is potentially less likely to resist, which minimizes the handling needed to ensure the procedure is carried out safely. This makes a passive fluid collector suitable for animals not used to human handling. Saliva contamination of the sample is potentially lower due to the involved mechanism. The valves are closed while being inserted via the oesophagus and remain closed until the handgrip is pulled. The latter should be done after the sampling probe is given enough time to sink completely in the rumen fluid. Without applying any vacuum, rumen fluid enters the sampling probe. After closing the valves using the handgrip and withdrawal of the OST device, the sampling probe can be unscrewed and emptied. The probe does not allow for large samples, on average 20–25 ml with a maximum of 40 ml [7]. The quality of the sample can only be assessed after complete removal of the OST device from the animal, increasing the chance for a second insertion if the quality is not deemed sufficient. As it is with all devices used in the OST technique, the exact location in the rumen is difficult to pinpoint, which is subject to variation. Further, there is a risk of saliva contamination during withdrawal of the OST if the valve does not close properly [3].

A vacuum pump builds up vacuum pressure in order to continuously collect rumen fluid after the sampling probe is fully submerged in the rumen content. An example of a vacuum pump fluid collector is the RUMINATOR sampling device (Profs Products, Wittibreut, Germany) [8]. This type of device is designed for collecting rumen fluid as, for example, performed for transfaunation of rumen fluid from one animal to the other. A vacuum pump is connected to a collection flask and, therefore, allows for large sample sizes (multiple litres) to be extracted and provides direct feedback on the sample quality. Saliva contamination might form a risk due to the saliva entering the sampling probe upon insertion into the oesophagus and due to the vacuum pressure if the sampling probe has not fully submerged. Also, obstruction of the holes in the sampling probe by small particles might increase the vacuum pressure needed to collect the fluid, extending the time the sampling probe has to stay within the animal. As with passive fluid collectors, the exact location in the rumen is difficult to pinpoint.

Several types of devices from different manufacturers can be used for sampling rumen fluid via the OST technique, all with specific advantages and disadvantages. The decision for the type of device should always follow the purpose of the rumen fluid collection and fit best with the type of ruminant, the familiarity of the animal with handling, and the amount of sample required.

Sampling guidelines

Below, the set of guidelines for collecting rumen fluid from intact ruminants using the OST technique is described in a step-by-step manner. Detailed information is given on the preparation, handling of the animal, insertion and removal of the OST device, obtaining a rumen fluid sample, and what to consider when sampling multiple animals. Additionally, remarks are given on the processing of the sample, solutions to common practical problems, and time of sampling relative to feeding.

A – Preparation before sampling

1. All instrumental parts coming into contact with the animal or rumen fluid (sampling probe, tubing, pump, collection flask) must be carefully cleaned before use. Preferably with disinfectant and thereafter thoroughly flushed with water.
2. Check all instrumental parts coming into contact with the animal or rumen fluid for mould, or other microbial growth, especially on sensitive parts like rubber rings or plastic tubing. Clean appropriately or replace the component.
3. Let all instrumental parts completely dry before re-assembling to avoid microbial growth.
4. When using a vacuum pump, check all seals and rings for damage and apply lubricant or replace where needed. Make sure, the collection flask can resist the applied vacuum when directly attached to the pump (i.e. using similar devices as the RUMINATOR).

5. Check the metal tubing and probe for any damages or loose particles and rigidity. The parts entering the animal should not form any risk in damaging the oesophagus or rumen wall and should not form a risk of detachment within the animal.
6. Set a marker on the tube on a standard length to ensure consistent insertion depths. The standard length should be appropriate for the ruminant species, breed, and life stage. The insertion depth of the OST is correlated to the animal size due to anatomical constraints; however, no standard relationships have been established. Some general measures used:
    - In adult Holstein dairy cattle (approximately 650 kg body weight, BW) the OST is inserted at 180–220 cm.
    - In sheep and goats (approximately 35–60 kg BW) a length of 120–150 cm and a diameter of 0.8 cm (0.6 cm inside and 0.2 cm wall thickness) are appropriate [6].
    - In calves between 2 weeks and 2 months of age, a 100 cm long softish tube (in diameter 0.8 cm inside/1.2 cm outside) can be used, without a OST sampling probe attached. The latter may cause some clogging problems, but the OST sampling probe is too large to be inserted in a calf.
    - In calves of 2 months of age, a 150 cm long tube (in diameter 1.1 cm inside/1.7 cm outside) can be used, without a OST sampling probe attached. The latter may cause some clogging problems, but the OST sampling probe is too large to be inserted in a calf.
    - In goats between 3 and 12 weeks of age, a 60 cm long tube with an internal diameter of 0.8 cm has recently been reported [9].
7. When working with young animals and different ruminant types, also take into account the diameter of the tubing and probe to be suitable for the size of the animal. Most standard devices can be run with different tubes to fit the target animal.
8. When working with a softish tube, one can consider to first insert a hard, slightly curved, tube in the mouth to keep the mouth open and to avoid the animal to chew on, and subsequently damage, the tube. Then the tube will be inserted through the hard tube into the animal.
9. The sampling probe and tubing can be pre-warmed in hand warm water to increase their flexibility and slippage. Hot water is discouraged as this might heat the metal parts too much and cause burn injury to the animal.

B – Handling of the animal and obtaining a rumen fluid sample

1. **Fixating:** The animal should be fixed, for example at the feeding fence or in a safety gate. Allowing for secure access from the front site, it is important to ensure that enough working space is available, to the sides as well.
2. For small ruminants, the person operating the probe could also hold the animal between the legs to better control the animal.
3. A halter can be used on the animal in extreme cases of resistance.
4. **Personnel:** All persons involved in handling the animal, inserting the tube and sampling probe, and collecting the fluid should be licenced according to local laws and regulations, and appropriately trained. Practise on a couple of animals, including all of the steps, is required before sampling for experimental purposes.
5. At all times, a minimum of two persons should be involved. One person should focus on the handling of the animal and can also insert the tube and sampling probe. A second person should focus on the collection of the fluid with the instrument and instantaneous sample processing.
6. When collecting rumen fluid samples from multiple animals with the same sampling device, an extra person should be dedicated to cleaning and drying the devices between animals (see 'C – Sampling multiple animals in one setting').
7. **Insertion and Depth:** Stand next to the head of the animal, with your back to the safety gate. An assistant can stand on the other side of the head.
8. When working with calm animals, pass the arm over the animal's head, while the side of the body is in contact with the head. Four of your fingers curl around the upper lip of the animal and pass through the diastema (the toothless part between the front teeth and the molars). The thumb rests on the nostril of the animal and the four fingers apply firm pressure on the hard palate. This will result in the animal keeping its mouth open. If the animal reacts against the procedure, the experimenter should not lay his/her arm around the head of the animal but instead use a halter to better control the head movement of the animal.
9. Make sure the throat, neck, and head are in one horizontal line with each other. **Do not** raise the head of the animal when inserting the probe.
10. Put the probe in the middle of the tongue and slowly insert it deeper into the mouth and

oesophagus. Stop with inserting at any moment when there is resistance. Give the animal time to swallow the probe independently.

11. When the animal heavily protests, it usually means that the person inserting the probe is too rough, or the probe is in contact with the trachea. Stop immediately, take out the probe and start over calmly. The insertion of the probe should always go smoothly.
12. Insert the probe and tubing until the pre-marked area is reached.
13. Deviations from the marked insertion depth do occur when performing the OST technique due to several reasons. Record the actual insertion depth when deviating from the theoretical insertion depth, and always report both values.
14. During the rest of the procedure, relax the grip on the animal a bit. Make sure the head of the animal is in a horizontal or downward line, never keep the head up. While having the probe inserted, it is more difficult to swallow for the animal and it will salivate more. The saliva must be able to easily leave the animal's mouth, otherwise it may flow into the trachea (i.e. risk for suffocation). This handling also helps to reduce saliva contamination in the rumen fluid sample.
15. Depending on the type of device being used, the sound and smell coming from the tube are important signs. No sound of ventilation should be heard, otherwise the probe is in the trachea. If the probe is positioned in the rumen, a typical rumen scent should be detected.
16. Depending on the type of the device being used, attach other parts to the tubing for collection of the rumen fluid.
17. **Sample collection:** Let the probe sink for approximately 20 s to increase the chance that the sampling probe fully submerges in the rumen fluid.
18. When using a passive fluid collector type of device, open the valves and wait for a couple of seconds for the fluid to collect in the probe. Close the valves, make sure the valves are completely closed before retrieval to avoid potential contamination.
19. When using a vacuum pump type of device, use the pump to build up the under-pressure and thereby collect the rumen fluid in a flask.
20. Sometimes little or no rumen fluid is collected when using the vacuum pump type of device. Retract the sampling probe and tube about 30–100 cm and reinsert again to the original depth, wait approximately 20 s before building up the vacuum pressure. Sometimes the probe gets clogged by particles or lays on top of a fibre mat, re-adjusting might help to overcome this problem. A too high under-pressure increases the risk of clogging. If the problem persists, it should be considered to completely remove the probe from the animal and use a reserve animal. The longer the probe is present in the animal, the more readjusting or reinserting causes cumulative discomfort for the animal. Also, check the device for faulty rubber rings or other seals, which prohibits the vacuum pressure build up.
21. When using a vacuum pump type of device, saliva contamination poses a greater risk. To minimise saliva contamination, it is therefore recommended to discard the first 50 ml of collected rumen fluid for small and young ruminants, and the first 500 ml of collected rumen fluid for adult cattle.
22. Saliva contamination can be checked on the consistency and colour of the rumen fluid collected. Saliva is colourless, sticky and difficult to pipet. When inserting a pipet in the fluid and taking it out again, saliva visibly sticks to the pipet. If needed, discard more than the 50 to 500 ml of collected rumen fluid as mentioned above, until the rumen fluid collected is of desired quality (i.e. minimal saliva contamination).
23. Collect a second or third batch as this will be a more representative rumen fluid sample.
24. **Retrieval of the probe:** Steadily and slowly retract the sampling probe and tube after enough rumen fluid has been collected. When using an electrical vacuum pump, switch-off the pump first before removal.
25. Always keep the head of the animal in a horizontal or downward position when removing the probe and tube. Do NOT keep the head of the animal upwards.
26. Check the animal for signs of damage. There should be no blood present on the device, in the mouth or saliva of the animal. Also, the animal should show normal signs of eating or rumination behaviour within 30 minutes after sample collection, even when still attached to the safety fence.
27. Thoroughly clean and inspect the sampling probe, tube and the rest of the device as described in ‘A – Preparation before sampling’.
28. When sampling multiple animals, follow the steps as described in ‘ C – Sampling multiple animals in one setting’.

C – Sampling multiple animals in one setting

When sampling multiple animals to collect a pooled sample, for example for *in vitro* incubation experiments, clean the outside of the probe and tubing of saliva, particles and traces of rumen fluid attached to it. The inside and outside should be flushed with water to reduce infectious cross-contamination between animals. When sampling multiple animals to collect individual rumen fluid samples for separate analysis, for example pH, VFA, other metabolites or microbial analysis, a thorough cleaning of the inside and outside is needed. The devices should be thoroughly cleaned between animals as described in 'A – Preparation before sampling ', including the complete drying of all instrumental parts. Due to practical constraints, like sampling multiple animals within a relatively short time frame (no time to completely follow the cleaning procedure described above), it is also recommended to work with multiple OST devices. If that is not possible, the following guidelines should be followed until further insight in the effects of cleaning between animals are available.

1. Clean the outside of all instrumental parts that comes into contact with the animal or rumen fluid under a constant stream of water, or use a fresh water bath. Warm water is recommended, usage of soap or other cleaning agents is discouraged due to potential residues. Hot water is discouraged, because this might heat the metal parts too much and cause burn injury on the animal.
2. Pay special attention to the holes in sampling probe and remove any particles obstructing them. Check the functionality of the valves, if present.
3. When using a passive collection system, detach the probe from the tube and rinse the inside with warm water. Also, clean the inside of the tube by passing through warm water multiple times if possible.
4. When using a vacuum pump system, clean the inside of the sampling probe and tube by pumping warm water multiple times through the complete system until only clean water is collected. If possible, the tube can also be connected to a (warm) water tap that provides sufficient pressure to flush the inside of the sampling probe and tube.
5. Make sure to use a freshly cleaned collection flask for every animal. Collection flasks can be re-used during the same experimental sampling if cleaned thoroughly with warm water and scrubbing. For practical reasons it is advised to have multiple collection flasks to easily swap between animals and allow time to thoroughly clean each flask before re-using.
6. Ensure there is no water left inside the probe or collection containers to avoid dilution of samples.
7. Make sure the clean device does not come into contact with other animals, feed particles or excreta to avoid contamination.

D – Processing the rumen fluid sample for different purposes

To increase comparability and repeatability of the performed research, detailed information on the processing of the sample should be given. Important details to report are: main purpose of collection (e.g. *in vitro* gas production, pH measurements, metabolite or microbial analyses), sample size (in ml), addition of preservatives (if applied), extra steps such as cooling (e.g. on ice), filtration, homogenization (e.g. stirring), time between sample collection, sub-sampling, processing and freezing, freezing method to directly stop microbial processes (e.g. liquid nitrogen, dry ice or −80°C freezer), temperature during storage and time of storage.

E – Final remarks to the time of sampling relative to feeding

The described set of guidelines above aims for standardization of the OST technique and therefore increased comparability and repeatability of research involving rumen fluid sampling of intact ruminant. Having said that, one should be aware that not only the method of sampling, but also time of sampling relative to feeding has a large impact on both the parameters measured in the rumen fluid and the ease of obtaining liquid rumen fluid [5], [6], [8]. Sampling before feeding allows for easier large liquid samples due to low amount of (fibre) particles, has a lower risk of saliva contamination and the sampling probe can be expected to reach more towards the cranial-central site and potentially more ventrally, because of a lower density of the fibre mat. Related to saliva contamination, in general 1h before and 3–4 h after feeding result in good quality samples with low saliva contamination. Sampling at feeding time and during the following 2 h has a higher risk of saliva contamination due to increased saliva production. These periods would vary depending on the type (e.g. proportion of concentrate and forage) of the diet fed to the animals.

The guidelines presented in this chapter are for general purposes and aim to assist researchers for optimal rumen fluid collection and to increase the comparability and reproducibility between research studies. The purpose of rumen fluid collection and the accompanying research question should always be leading, therefore deviations on these guidelines are possible.

References

1. Duffield T, Plaizier JC, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J, McBride B. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J Dairy Sci. 2004;87(1):59-66. DOI: 10.3168/jds.S0022-0302(04)73142-2
2. Henderson G, Cox F, Kittelmann S, Miri VH, Zethof M, Noel SJ, Waghorn GC, Janssen PH. Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. PLoS One 2013;8(9):1-14. DOI: 10.1371/journal.pone.0074787
3. Larsen M, Hansen NP, Weisbjerg MR, Lund P. Technical note: Evaluation of the ororuminal FLORA sampling device for rumen fluid sampling in intact cattle. J Dairy Sci. 2020;103(1):447-50. DOI: 10.3168/jds.2019-16972
4. Shen JS, Chai Z, Song LJ, Liu JX, Wu YM. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. J Dairy Sci. 2012;95(10):5978-84. DOI: 10.3168/jds.2012-5499
5. Wang M, Wang R, Janssen PH, Zhang XM, Sun XZ, Pacheco D, Tan ZL. Sampling procedure for the measurement of dissolved hydrogen and volatile fatty acids in the rumen of dairy cows. J Anim Sci. 2016;94(3):1159-69. DOI: 10.2527/jas.2015-9658
6. Ramos-Morales E, Arco-Perez A, Martin-Garcia AI, Yanez-Ruiz DR, Frutos P, Hervas G. Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats. Anim Feed Sci Technol. 2014;198:57-66. DOI: 10.1016/j.anifeedsci.2014.09.016
7. Geishauser T, Linhart N, Neidl A, Reimann A. Factors associated with ruminal pH at herd level. J Dairy Sci. 2012;95(8):4556-67. DOI: 10.3168/jds.2012-5380
8. Geishauser, T. An instrument for collection and transfer of ruminal fluid and for administration of water soluble drugs in adult cattle. Bov Pract. 1993;27:38-42. DOI: 10.21423/bovine-vol1993no27p27-42
9. Belanche A, Palma-Hidalgo JM, Nejjam I, Jimenez E, Martin-Garcia AI, Yanez-Ruiz DR. Inoculation with rumen fluid in early life as a strategy to optimize the weaning process in intensive dairy goat systems. J Dairy Sci. 2020;103(6):5047-60. DOI: 10.3168/jds.2019-18002

Corresponding authors: Sanne van Gastelen, Wageningen University & Research Wageningen, Niederlande, E-mail: sannevangastelen@gmail.com

Citation note: Muizelaar W, Bani P, Kuhla B, Larsen M, Tapio I, Yáñez-Ruiz D, van Gastelen S. Rumen fluid sampling via oral stomach tubing method. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, Dewhurst R, Ferguson H, Terré M, Kuhla B, (editors). Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2020-. DOI: 10.5680/mcpb008