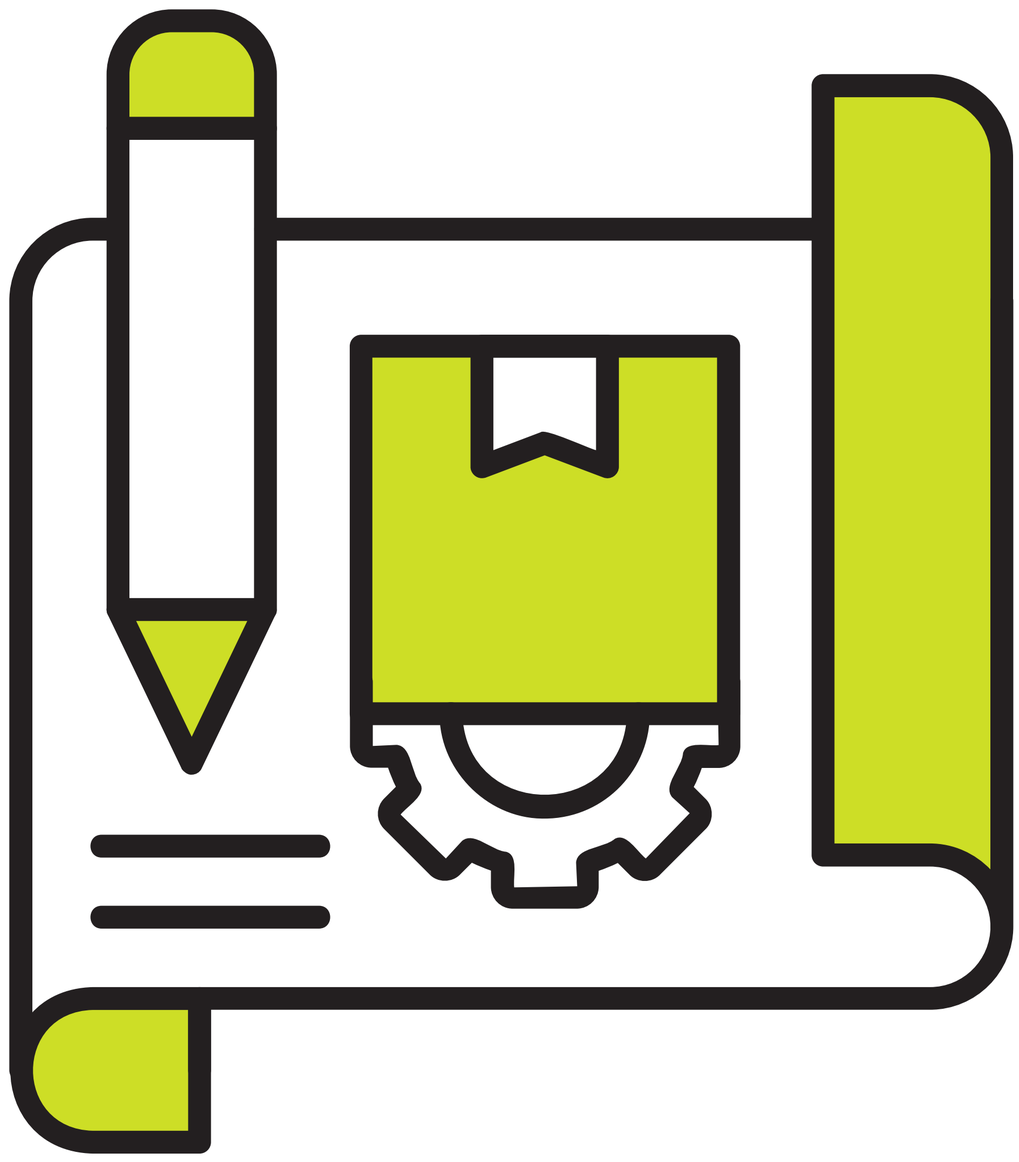
HoloRuminant
Understanding microbiomes of the ruminant holobiont

HOLORUMINANT PROTOCOL

Protocol for Rumen Fluid Sampling – Flora Rumen Scoop



Authors: Joana Lima, Christopher Creevey, Milka Popova, Ignas Silinskas

Version: V1

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement № 101000213

PROTOCOL FOR RUMEN FLUID SAMPLING – FLORA RUMEN SCOOP

Materials needed:

- Flora scoop
- Stomach tub
- Sterile 20ml container
- Sterile 2ml tubes
- Gloves

Note: Withhold feed from animals for minimum of 2 hours prior to sampling. This is to limit the amount of feed particles that will be present in the fluid sample. The abundance of different microbial groups will alter depending on when the rumen sample is collected.

1. Restrain the animal, assuring it is not overly stressed and maintaining a comfortable body position to avoid injury. Depending on animal behaviour, the animal's head may need to be tied. If so, ensure the head is pointing skyward and the neck extended without forcing to allow passage of the tube. Additionally, a head lift may also be used.

2. Ensure the holes of the Flora Scoop are fully closed above the cup section of the stomach tube.

3. Insert the stomach tube into the mouth. Do not restrict the animal's lower jaw/tongue movements - facilitates the "swallowing" of the tube i.e. hold the animal by the upper jaw. Do NOT hold the animal by the nose. Ensure the tube is kept in the middle of the mouth / over the tongue to minimise biting. If there is resistance, it may be a sign that the tube is not properly positioned.

4. Gently push the stomach tube down into the rumen until resistance is met. Retract the tube slightly to aid positioning in the rumen.

5. Open the cup.

6. Let rumen fluid enter the cup by waiting for 40 to 60 seconds.

7. Close the cup.

8. Withdraw the stomach tube slowly and steadily from the rumen/tract.

9. Unscrew the cup and pour the juice into the 20 ml sterile container.

10. Aliquot rumen fluid by pipetting 0.5 ml rumen fluid into each 1.5- or 2 ml sterile tube.

11. Label the tubes, including animal identification number, type of sample, and date.

12. Snap freeze samples in liquid nitrogen. Be sure to leave space at the top of the tube to allow liquid to expand when frozen. Sealed tightly the tubes to prevent leakage. Ensure that the liquid nitrogen is handled with care to avoid injury. Also, wear protective gloves and goggles when handling liquid nitrogen.