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INNOVATED SURGERY PROTOCOL FOR RUMEN CANNULATION IN RUMINANTS

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ABSTRACT

The manuscript describes surgical technique of rumen cannulation in cattle using flexible cannulas, which are necessary in prediction of experimental feeds' potential degradability using an *in situ* method. The main principles of pre-operative preparation of the experimental animals and their post-operative treatment to ensure their long-term use in experiments with no complications are also discussed. In addition, we provide a brief overview of the history of surgical techniques and procedures used to determine feed digestibility. This overview proves that, despite the efforts to avoid surgeries on experimental animals, these remain actual and necessary.

Key words: ruminants; surgery; rumen cannula; pre- and post-operative treatment; *in situ*

INTRODUCTION

Cannulated ruminants are irreplaceable in evaluation of potential feed degradation, which is determined by the *in situ* method, and in combination with duodenal intestinal cannula it creates the basis to determine the intestinal-enzymatic or total digestibility of feed (Chrenková *et al.*, 2012; 2018). In the past, firm PVC cannulas were used for rumen cannulation, which were composed of several components and were completed during the surgery itself. At present, compact flexible cannulas (patent Bar Diamond Inc., USA) are used. These differ in their diameters, the length of the tube, and diameters of the fixation bases depending on the size of the animal. The flexible cannula requires some changes in the already described cannulating process (Szakács *et al.*, 1990a; b). We consider it important to name the medications available and used at present rather than those, which are no longer manufactured. We updated also the older surgical techniques used in rumen cannulation.

MATERIAL AND METHODS

Rumen cannula

For the surgery, a rumen cannula for small ruminants produced in Bar Diamond, Inc. from the USA (Figure 1) was used. Its description and composition are at the website https://shop.bardiamond.com/en/small-rumen-cannulae (# 8C). Later, it was replaced by a cannula for large ruminants from the same producer https://shop.bardiamond.com/en/large-rumen-cannulae (# 1C).

Preparation of animals

Animals were selected basing on the aim of the research, with the goal of their long-term

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 # 8C **# 1C**

Figure 1. Rumen cannula

utilization. Most suitable are young heifers (up to 250 kg), which have low amounts of subcutaneous and deposit fat, making the surgery much easier. At the first phase, it is necessary to acclimate the animals to tied-up housing as well as daily physical contact in the region of *Fossa paralumbalis*. This daily "training" combined with feeding, cleaning and washing of the animals continues after the surgery as well, with a pause to allow healing of the surgical wound. The experimental animals then do not develop negative association in relation to the short-term pain after the surgery, therefore, later it is not necessary to sedate or fixate them when manipulating the cannula during the experiments. A pre-surgical diet (hay for 48 hours) is necessary when cannulating an animal. Hay and water cease to be provided only in the morning of the surgery. Filled rumen after the evening feeding maintains an almost physiological position of this organ, which makes the surgery considerably easier.

The surgery itself consists of several stages:

Preparation of the surgical site (after shaving of the region of *Fossa paralumbalis* the previous day) consists of mechanical cleaning, degreasing of the surgical field (Benzinalcohol) and then disinfection using Ajatin tincture. The night before the surgery, the ruminal cannula is placed into clean water with Ajatin solution.

Sedation and anaesthesia. Sedation using xylazin (Rometar 2 % inj.) in the amount 0.10 – 0.15 ml.100 kg^{-1} of live weight. Xylazin is characterised, in addition to its analgesic and myorelaxant properties, also by its effect on the motor functions of the digestive tract, therefore, we recommend a minimum dose necessary to calm down and immobilize the animal. High doses may cause not only the animal to lie down but a risk of long-term atony of the foreguts. Therefore, we ensure the quality of the local anaesthesia using 2 % solution of procaine (Procain Bioveta 100 mg.100 ml^{-1} inj., with the effective substance Procaini hydrochloricum 100 mg) at the maximum amount of 60 ml, starting with the subcutaneous layer, through all muscle layers and around the perimeter of the presumed circular incision. After uncovering the peritoneum, a few drops may be applied on its surface as well. Sufficient local anaesthesia is reached in 15 minutes. Before initiating the surgery, the antibiotic Norostrep inj. or Shotapen inj. at recommended doses is applied.

The circular resection of the stomach cavity is always performed after placing a sterilised tube of the cannula, coated with pyotanicin (crystal violet, methylrosanilinii chloridum) to the centre of the left *Fossa paralumbalis*. The incision into skin (cutis) is led

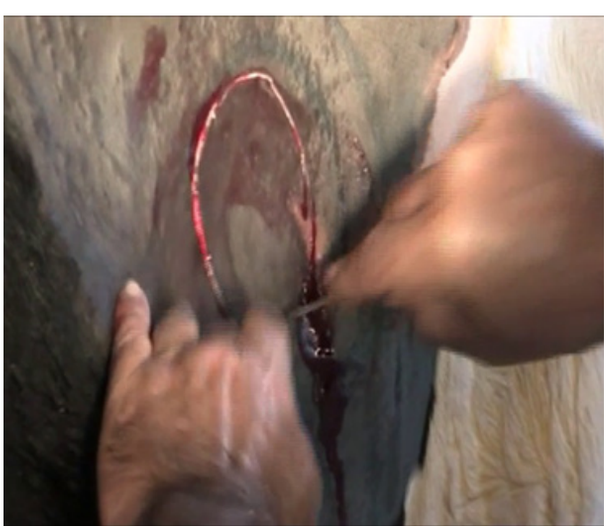

Figure 2. Incision through the skin

along the inner perimeter of the marking (Figure 2). Further, to proceed in the following order: subcutis, tunica flava abdominis and muscle layers (m. cutaenus trunci, m. obliquees externus et internus abdominis, m. transversus abdominis). Veins are ligated with each layer to prevent bleeding into the stomach cavity after peritoneum is cut. After peritoneum is pulled out using a haemostat, we cut through it under hand control using blunt scissors. The muscle and peritoneum should be preserved as much as possible. Using this way, later enlargement of the created stoma by the cannula's weight is prevented.

The uncovered rumen is fixed using haemostat in the wound in the way to avoid larger branches v. ruminalis sinister and prevent damage to branching of vegetative nerves. It is also important to preserve the topography and avoid the entry of the inner disc of the cannula outside the dorsal ruminal sac. If the rumen is almost full, it is lifted directly into the surgery wound and the necessity of fixation is eliminated.

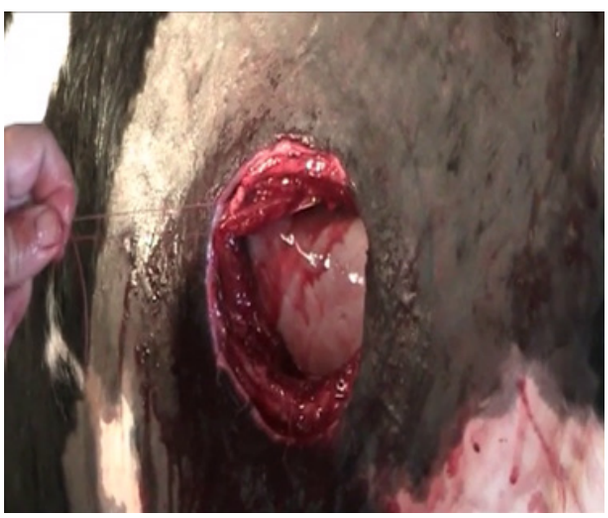

Figure 3. Suturing the rumen wall with the abdominal wall

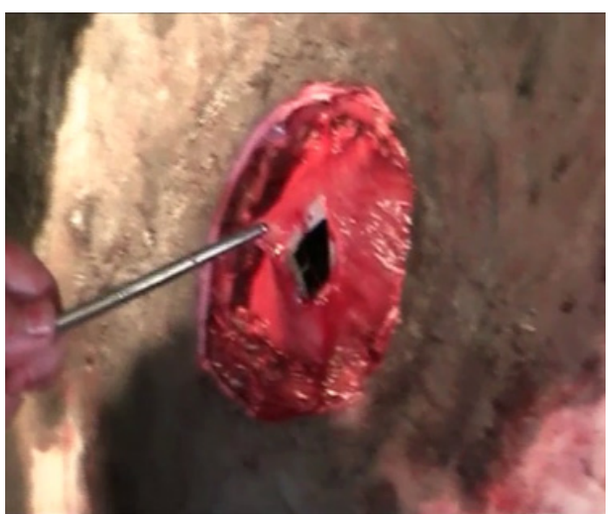

Figure 4. Opening of the rumen after pressurization of the abdominal wall

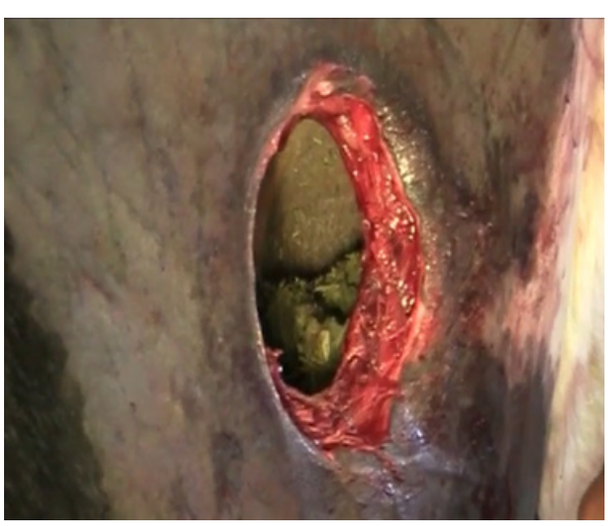

Figure 5. Rumen prepared for insertion of the cannula

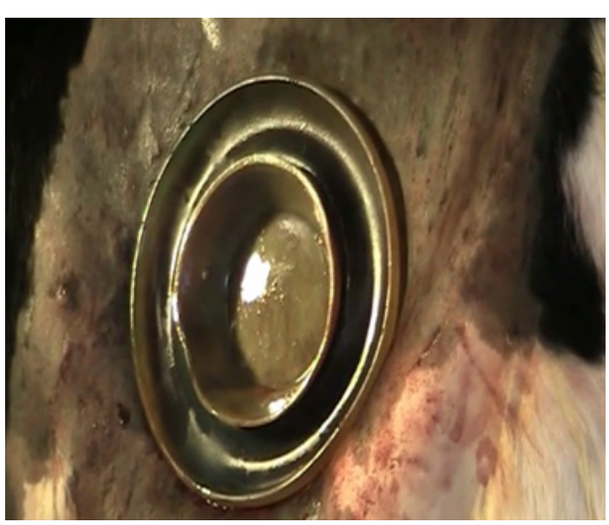

Figure 6. Completed rumen cannula, corked

After hermitization of the stomach cavity, the next phase is dressing of the rumen wall approximately 3 – 4 mm above the sutures, which allows sufficient space for later ligation of veins (Figure 4). This is the phase with the strongest bleeding, even if a site without visible larger veins was selected. The bleeding veins need to be carefully ligated, since the flexible cannula does not provide pressure compression as was the case with tightened disks of a firm cannula.

After treating the dried and cleaned wound with antibiotic ointment (Figure 5), the cannula itself is inserted by deforming the inner fixation disk. The position of the inner disk and its re-straightening in the dorsal ruminal sac is evaluated manually through the tube of the cannula and the tube is corked (Figure 6).

After the surgery, a dose of analgesics (Novasul inj., Richterpharma AG) is administered to prevent the cannula being ripped out by defensive reactions after the local anaesthetics fade. The analgesics are administered for several days after the surgery, as needed, which lowers the post-operative stress and, therefore, speeds up the healing of wounds as well as prevents defensive reactions of the cannulated animals during handling.

During a long-term use of the experimental animals, vitamin injections (ADE-Vit a.u.v. inj., Bioveta, Ivanovice na Hané) are administered regularly; for prevention of liver damage, Menbuton "WERFT" (Sanochemia Pharmaceutika AG) is applied.

Post-operative care

For 2 days after the surgery, quality hay is provided until the atony of rumen, caused by Xylazin, passes entirely. Afterwards, acclimatisation to experimental diet begins (minimum of 14 days). Antibiotics (Norostrep inj.) are administered for 4 – 5 days after the surgery, resp. on the third day after two administrations of Norostrep Shotapen inj. is depot delivered. In case of oedema, it is treated using resorption ointment (Aphlegmin ung.). Skin sutures are not removed; they usually loosen during the healing period through maceration of stoma by the tube when the animals move and by re-established contractions of the rumen. Between the 8th and 10th day, the cannula is removed from the rumen and the rumistoma is examined visually and by palpation. In case of bags, those are treated with antibiotic ointment. Necrotic tissue (remnants of sutures, peritoneum above the sutures) is removed. After cleaning the skin, Chlorophylum spray is applied. The cannula is inserted again and corked. To protect the skin at the site of cannula's entry, dermatologics (Infandolan ung., Indulona) in the form of ointments are applied permanently in order to prevent maceration by leaking rumen fluid. We consider important the daily physical contact of the treating staff in the location of cannula. This "training" completely eliminates later defensive movements and stress of the animal. Using this procedure, the animals are usable for experiments without obstacles for several years.

RESULTS AND DISCUSSION

Technique of rumen cannulation in large and small ruminants has been known for decades (Dougherty, 1965). This surgical technique, with small adjustments, is still used today. Various modifications of surgical procedures and the options to utilize different types of cannulas have been described in detail (Szakács *et al.*, 1990a; b), however those had been always technical aspects and details related to the surgery itself, which were meant to minimalize post-operative complications, secure anaerobic environment in the rumen and, therefore, enable a long-term use of cannulated animals in experiments.

In contrast to Aliev (1974), we did not lead the knots of the sutures of the abdominal wall with rumen through the skin, because we are of the opinion that such sutures covered by the outer disc of the cannula and without air could begin to fester. In case of post-operative oedema, they would also be at risk of tearing. Despite the loss of some portion of muscle mass in the use of a circular incision through the abdominal wall, we rejected also the procedure suggested by Němeček *et al.* (1981). When a horizontal incision through the skin was made, it was necessary to suture it after the surgery, which caused the risk of inflammation of the surgical wound at the lower edge after contact with the secretion from the wound or the rumen fluid containing microflora. In general, attempts to divide the surgery into two stages have also been abandoned, but this option is still being tested (Malik et al., 2015). Size of the rumen cannula and its width depend not only on the type of animal

but its weight as well. We prefer cannulas the tube of which allows access by hand (8 cm), which are replaced with larger ones (12 cm) as the animal grows. Corks are provided with a fixation apparatus to secure bags.

It can be stated that the surgery itself has always been among the simplest in the experimental veterinary surgery practice, but from the aspect of maintaining the function of the operated organ, which can be compared to an anaerobic fermenter, also the most complicated.

Selection of cannula is determined by methodical aims. When rumen fluid needs to be collected for experimental and study purposes, a simple thin cannula is sufficient, but collection of the rumen content requires a tube of a larger size, which would allow an entry by hand. The principle is to minimalize the invasiveness of the surgery on the animal while allowing to achieve goals of experiments.

To clarify, it is necessary to return into the history of development of new testing methods for feed digestibility. *In vivo* methods of balance experiments and marker experiments were intensive in terms of time, labour and costs (Dhanoa *et al.*, 1985). In parallel to searching for cheaper alternatives, the resistance against experiments on animals was also growing. In the 1960s, an *in vitro* method was developed by Tilley and Terry (1963) and it entered mass use, during which it was also being improved upon and factors that could affect the results were also tested (Daniel, 1984; Chrenková, 1984). At the same time, the results obtained by this method were compared to the results gained from *in vivo* experiments (Shqueir *et al.*, 1984). The aim was to develop an artificial rumen (RUSITEC) and the animals would become only donors of rumen fluid with its specific microflora and microfauna (Brice and Morrison, 1984). These authors tried to eliminate the main disadvantage of the *in situ* method: the real processes in the rumen cannot be described using a static model. Rusitec as a dynamic system removed problems with growing mass of bacterial protein, which limited the length of incubation of the experimental material in given environment.

In the 1980s, with the growing interest in temperature and physical methods of treatment to protect plant proteins from their high degradability in rumen, the *in vitro* method showed its limitations. Although it allowed to test the effect of the used treatment on degradability of proteins in rumen, it was impossible to determine, whether these "bypass proteins" affected by Maillard's reaction or higher doses of chemicals in treatments are actually digested by enzymes in the intestines. Developing an *in situ* method to determine rumen degradability of nutrients required a return to the use of cannulated animals (ØRSKOV *et al.*, 1980; Hunter *et al.*, 1981). Animals with a combination of rumen and intestinal cannulas enabled to determine not only the potential degradability of nutrients but their intestinal and total digestibility as well. For this purpose, the mobile bag method was developed (Szakács, 1989). Even in this case, to simplify the method and for the benefit of the animals, the polycannula method (cannulas in duodenum, abomasum and ileum) was abandoned in favour of animals with only rumen and duodenal cannulas. This variant is still successfully used today (Chrenková *et al.*, 2012; 2018).

This short excursion into the history of the method, although it is not directly related to the topic of this manuscript, was not purposeless. It proves that despite the attempts to avoid surgeries in experimental animals, these remain actual and necessary. In addition to limiting the demands for material and labour, all operations on animals are performed *lege artis* and in accordance to ethical principles, with the aim to minimalize the scope of surgeries, the pain through analgesics before and after the surgery and to ensure the maximum comfort of the animals during the experiments.

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