A Method for Isolation of the Ruminoreticulum in the Cow

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In order to investigate the role of the rumen in the net absorption of water, electrolytes and volatile fatty acids (VFAs), a reliable method to isolate the rumen is essential. Though a number of methods have been devised [1, 2, 4, 5], most of them are costly in terms of labor time and special instrumentation, and furthermore, not reliable in the evaluation of total absorption of the rumen in physiological status. Among them, Martens' method used in a sheep study has advantages that the epithelium is bathed with rumen contents just prior to the experiment and that animals can be used repeatedly [4].

In this paper, we have devised a more practical and reliable method for isolation of the ruminoreticulum in the cow by modifying the Martens' method, and confirmed high reliability and reproducibility of the method.

Experimental animals: Four one-year-old Holstein heifers were surgically fitted with a permanent rumen fistula (10 cm in diameter) 2 months before the experiment. They were kept in individual stalls and fed 2.5 kg of alfalfa hay and 1 kg of concentrate twice daily. The body weights of the heifers were 258–305 kg at the beginning of the experiment.

Procedures to isolate the ruminoreticulum: The isolation was performed through the rumen fistula as follows. The animal was restrained in a stock. The rumen was completely emptied and washed twice with 10 l of 150 mM NaCl at 38°C. For the isolation of the ruminoreticulum from the omasum and the abomasum, the plug made of polyethylene foam (Fig. 1) was inserted into the entrance of the abomasum through the reticulo-omasal orifice. To avoid drainage of saliva into the rumen, a balloon catheter (10 mm outer diameter) was placed at the caudal esophagus and the saliva introduced into a collecting bottle from the balloon catheter was continuously infused directly into the abomasum through a silicone tube (15 mm outer diameter) penetrating

the plug (Fig. 2). The settlement of the balloon catheter in the esophagus was carried out by withdrawing a polyethylene catheter (2.5 mm outer diameter), which was inserted from the nose into the rumen and fastened to the balloon catheter at the end. Then the balloon was inflated to a pressure of about 150 mmHg. Before the experiment, the rumen was washed again with 10 l of NaCl solution.

Reliability of the isolation procedures: To estimate the reliability, the leakage rate of saliva into the ruminoreticulum and of the test solution from the ruminoreticulum were studied. Thirty or 40 l of the test solution containing certain concentrations of electrolytes and VFAs (100–500 mOsm./l, pH 6.8) was poured into the isolated ruminoreticulum. To evaluate the isolation, polyethylene glycol (PEG), non-absorbable marker, was added to the solution at a concentration of 1.5–3.0 mg/ml. Three hours later, the whole volume of the solution was removed from the ruminoreticulum, and the recovery rate of PEG was determined [3].

To estimate the contamination of saliva to the ruminoreticulum, creatinine (10 mg/ml) was infused into the saliva in the esophagus at a flow rate of 300 ml/hr through the nasal catheter.

Fig. 1. The cannon ball-shaped reticulo-omasal orifice plug made of polyethylene foam. The length of the plug is about 20 cm and the diameters are about 8 cm at the top and the end and about 6 cm at the center, respectively.

Fig. 2. Schematic figure of the ruminoreticular isolation. A: Reticulo-omasal orifice plug, B: Ballon catheter, I: Rumen, II: Reticulum, III: Omasum, IV: Abomasum.

After 3 hour isolation, the creatinine level of the solution was determined. The rumen fistula was closed with a cork plug throughout the experiment. Each animal was used at 2 week intervals and totally 18 isolation studies were performed.

The mean recovery rate of PEG in the solution was $95.4 \pm 5.8\%$ with a range of 83.0–104.1%. Creatinine was detected in only 5 out of 18 experiments, and the mean detected creatinine level was 1.9% (0.6–4.6%) of total infused creatinine. These results indicated that the leakage from the isolated ruminoreticulum and the contamination of saliva were quite minimal.

Reproducibility of the isolation procedures: This isolation method was applied to 2 heifers 6 times at 2 week intervals, and the reproducibility of the recovery rates of PEG and the contamination rates of creatinine were studied in a same way as that for reliability. The PEG recovery rates were maintained high in all the trials and the contamination of saliva was found only twice in a heifer (Table 1). This suggests that the damage to the ruminoreticulum, esophagus and reticulo-omasal orifice by these isolation procedures is very small. These procedures sometimes

Table 1. PEG recovery rate and creatinine contamination rate in repeated trials of the ruminoreticular isolation procedures. (—): represents a contamination rate (%) of creatinine.

	Animal No.	Trial					
		1st	2nd	3rd	4th	5th	6th
PEG recovery rate (%)	2	95.2	104.1	101.0	85.2	95.0	100.0
	4	83.0	99.1	100.2	94.5	97.2	96.4

induced the sign of distress or discomfort in the cow. But, they seemed to be accustomed to the procedures after a few trials and showed no abnormal behaviors with normal ruminal contraction.

From these results, this isolation method is concluded to be quite reliable and practical for the study on the role of bovine rumen in net absorption of fluids and various materials.

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要 約

牛の第一・二胃の分離法（短報）：田原秀樹・池田 賢・佐々木伸雄・竹内 啓（東京大学農学部家畜外科学教室）——第一・第二胃壁における水分，電解質，VFAの絶対移動量を測定するための両胃分離法について検討した。第二・三胃口にはポリエチレン製プラグを装着して第一胃内容の漏出を防止し，食道内にはバルーンカテーテルを留置して唾液流入を防止したところ，第一・二胃の分離は良好であり，生体に与える影響も少なく，動物は繰り返しの実験に十分堪えた。