

< Original Article >

Effects of rumen cannulation surgery on physiological parameters and rumen fluid pH in Korean native Hanwoo cattle

Eunju Kim¹, Seong Bum Kim¹, Youl Chang Baek², Min Seok Kim², Changyong Choe¹,
Jae Gyu Yoo¹, Younghun Jung¹, Ara Cho¹, Suhee Kim¹, Yoon Jung Do^{1*}

¹*Division of Animal Diseases & Health, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea*

²*Animal Nutrition and Physiology Team, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea*

(Received 4 July 2018; revised 28 September 2018; accepted 30 September 2018)

Abstract

Rumen cannulation is used for nutritional and microbiological research, clinical diagnosis, and rumen component transfaunation. However, the cannulation procedure can affect parameters such as complete blood count findings, serum chemistry, and rumen fluid pH. The objective of this study was to evaluate the health risks related to the rumen cannulation procedure over a 1-month period. We did not identify significant differences in red blood cell numbers or morphologies between pre- and postoperative timepoints. Moreover, no inflammation or infection was detected. Despite the absence of apparent clinical signs after surgery, serum chemistry results revealed changes in blood urea nitrogen levels and the activities of liver enzymes, including aspartate transaminase, lactate dehydrogenase, and creatinine kinase, from postoperative days 1 to 14. Rumen fluid pH, as measured from samples collected via an orogastric tube, was slightly increased after a preoperative fasting period and on postoperative day 1 but decreased thereafter from postoperative day 4, indicating a minor influence of cannulation surgery on ruminal fluid pH. This is the first study to evaluate hematological parameters and rumen pH before and after rumen cannulation surgery in Hanwoo cattle. Further research is required to better elucidate the potential effects of rumen cannulation surgery on animal health.

Key words : Cattle, Orogastric tube, Rumen cannulation, Rumen pH, Liver enzymes

INTRODUCTION

Rumen fluid analysis is used not only for the clinical diagnosis of acute, subacute, and chronic ruminal acidosis (Alonso, 1979; Duffield et al, 2004; Perumbakkam and Craig, 2012) but also for research regarding the function and activity of the ruminant forestomach and microflora (Ramos-Morales et al, 2014; Artegoitia et al, 2017; Noel et al, 2017; Ribeiro et al, 2017) and associated metabolic abnormalities (Terré et al, 2013).

Ruminal pH is an important indicator of overall health and digestive function, including absorption, nutrient utilization, and carbohydrate metabolism (Kleen et al, 2004; Yilkal et al, 2016). Ruminal fluid is commonly collected via rumenocentesis, rumen cannulation, or an orogastric tube (Leek, 1976; Duffield et al, 2004; Kleen et al, 2004; Laflin and Gnad, 2008; Ramos-Morales et al, 2014). Of these methods, an orogastric tube is the least invasive, but samples from the ororumenal probe are readily contaminated with alkaline saliva during passage through the esophagus, pharynx, and oral cavity (Duffield et al, 2004; Kristensen et al, 2010; Terré et al,

*Corresponding author: Yoon Jung Do, Tel. +82-63-238-7222,
Fax. +82-63-238-7235, E-mail. clonea@korea.kr

2013). Because of these limitations, orogastric sampling often results in the mismeasurement of rumen pH. As an alternative, rumen cannulation surgery is being increasingly used for rumen component collection in scientific research (Leek, 1976; Laflin and Gnad, 2008; Ramos-Morales et al, 2014). This procedure collects more fluid and prevents salivary contamination. Rumen cannulation allows the continued monitoring of rumen pH parameters via repeated sampling throughout the day (Terré et al, 2013; Kiro, 2017), but it requires surgical preparation and an aftercare period of at least 3 weeks and is likely to induce pain and stress (Ramos-Morales et al, 2014; Ribeiro et al, 2017). Accordingly, the procedure can affect the measured rumen pH parameters such as pH, volatile fatty acids, and lactic acid, which are related to microbial populations in the rumen fluid (Grofum, 1989; Terré et al, 2013). For cattle undergoing rumen component transfaunation or used in research, it is critical to understand the features of rumen recovery time after rumen cannulation (Leek, 1976; Sato, 2016). Additionally, previous reports have identified some risks associated with rumen fistula surgery, including rumen fluid leakage, infection, inflammation, hematoma, abscess of the puncture site, and peritonitis (Strabel et al, 2007; Durmic et al, 2015; Rafee et al, 2015; Sato, 2016). These complications can severely affect measured results, such as complete blood counts (CBCs), serum chemistry, and rumen fluid pH (Rafee et al, 2015; Sato, 2016).

The objective of this study was to evaluate the health risks associated with rumen cannulation for up to 1 month after the procedure. We examined longitudinal changes in physiological parameters including rumen pH between pre- and postoperative timepoints in Hanwoo cattle, a native Korean breed, that underwent rumen cannulation surgery.

MATERIALS AND METHODS

Animals

Four male Hanwoo cattle (15 months old, mean body weight: 300 kg) were selected for the study. The cattle

showed no signs of clinical disease and were verified as disease-free (i.e., free of bovine tuberculosis, Johne's disease, foot-and-mouth disease, brucellosis, and bovine leukosis) before the experiment. Table 1 shows feeding information for Hanwoo cattle.

Rumen cannulation surgery

The cattle were fasted for approximately 24 hours prior to surgery. The cattle were sedated with 20–40 mg of xylazine hydrochloride (23.32 mg per 300 kg), and local anesthesia was induced with lidocaine HCl 2% (5 ml) injected subcutaneously and into the deep muscle layers surrounding the incision site. For preparation of the incision site, hair was removed, the skin was scrubbed with 10% povidone iodine solution 3 times, and general aseptic surgical procedures were followed. The forestomach was approached from the left paralumbar fossa. The location of the incision site was the ventral part of the lateral process between L1 and L3 and between the caudal part of the 13th rib and the point of the hip (i.e., the tuber coxae). A circular area of skin with a diameter equal to the inside diameter of the cannula was excised. The external abdominal oblique muscle fibers were bluntly dissected to expose the internal abdominal oblique muscles, which were then dissected. The transverse abdominal muscles were also dissected to reveal the peritoneum. The rumen wall was sutured to

Table 1. Ingredient composition of the total mixed ration for Korean native cattle (Dry matter basis)

Ingredient	Percentage (%) as feed
Ground corn	38.32
Wheat bran	32.80
Soybean meal	4.00
Rapeseed meal	1.60
Molasses	1.60
Rice straw	10.00
Corn silage	10.00
Dicalcium phosphate	1.20
Salt	0.32
Vitamin-mineral mix ^{a)}	0.16
Total	100

^{a)}Vitamin A, 2,650,000 IU; Vitamin D₃, 530,000 IU; Vitamin E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Fe, 13,200 mg; I, 440 mg; Co, 440 mg.

the skin with 4 interrupted horizontal mattress sutures. The rumen was punctured with a scalpel, and a portion was then excised. After the rumen had been sutured to the skin with chromic catgut, the exposed muscle and skin were rinsed with sterile saline, and a 4-inch rumen cannula (Bar Diamond, Parma, ID, USA) was then inserted into the rumen by advancing the conical flange through the rumen fistula. After cannulation, all cattle were surgically castrated and allowed to recover in a separate enclosure. The surgical site and outer rim of the cannula were examined on postoperative days 1, 2, 3, 4, 7, 10, 14, 18, 21, and 24.

Hematological parameters

Blood samples were collected via jugular venipuncture into ethylenediaminetetraacetic acid-containing tubes for CBCs immediately after surgery, and on postoperative days 1, 2, 3, 4, 7, 10, 14, 18, 21, and 24. Within 1 h of collection, whole blood samples were analyzed with an automated device (IDEXX ProCyt Dx Hematology Analyzer, IDEXX Laboratories, ME, USA) to measure red blood cell (RBC) counts; total white blood cell (WBC) counts; counts for WBC subtypes including neutrophils, lymphocytes, monocytes, eosinophils, and basophils; hemoglobin levels; hematocrit levels; mean corpuscular volumes; mean corpuscular hemoglobin concentrations; red cell distribution widths; and total platelet counts.

Serum chemistry parameters

For serum chemistry analysis, blood samples were collected into vacutainer tubes without anticoagulants for serum chemistry, immediately after surgery, and on postoperative days 1, 2, 3, 4, 7, 10, 14, 18, 21, 24, and 28, and serum was harvested by centrifugation at 3,000 g for 10 min at 4°C and stored at -20°C until analysis. All serum samples were analyzed for albumin (ALB), triglycerides (TG), glucose (GLU), creatinine kinase (CK), creatinine (CRE), gamma glutamyltranspeptidase (GGTP), aspartate transaminase (AST), lactate dehydrogenase (LDH), total bilirubin (T-BIL), total protein and blood urea nitrogen (BUN) with an automated biochemical

blood analyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Rumen fluid collection and pH measurement

Rumen fluid was collected via an orogastric tube and immediately subjected to pH measurement in triplicate with a commercial pH meter (SevenEasy pH, Mettler-Toledo, Greifensee, Switzerland). Two days before surgery, the first rumen sample was collected at 10:00 AM, and the cattle were then fed at 10:30 AM and 4:00 PM. The day before the surgery, the cattle were fasted in order to prevent intraoperative contamination from stomach contents and to minimize food intake-related rumen pH measurement errors. On the day of the surgery, a preoperative rumen fluid sample was collected at 10:00 AM. The surgery itself was conducted from 10:00 AM to 10:30 AM, and the first postoperative feeding occurred at 4:00 PM. Beginning on postoperative day 1, the cattle received daily feedings at 10:30 AM and 4:00 PM. Rumen fluid samples were collected at 10:00 AM on postoperative days 1, 4, 7, 10, 14, 18, 21, 24, and 28.

Statistics

We analyzed the effects of rumen cannulation surgery on hematological parameters and rumen fluid pH over the first postoperative month. The statistical analysis was performed with SPSS software version 21 (IBM, Armonk, NY, USA). The analyzed data were visualized with GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA, USA). For all analyses, $p < 0.05$ was the threshold for statistical significance.

RESULTS

Rumen cannulation surgery had no adverse effects on the cattle and no apparent effects on their drinking and eating behaviors. Postoperative physical examinations did not reveal any clinical signs, such as inflammation or infection.

Table 2. Average hematological values in Korean native cattle over the first postoperative month (mean±standard deviation)

	RBC (10 ⁶ /μL)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	WBC (10 ³ /μL)	Ne (10 ³ /μL)	Ly (10 ³ /μL)	Mo (10 ³ /μL)	Eo (10 ³ /μL)	Ba (10 ³ /μL)	PLT (10 ³ /μL)
Be S	9.03±1.70	12.58±1.62	38.30±6.26	42.63±2.90	14.08±1.02	32.95±1.20	35.03±3.81	10.01±1.72	3.26±1.20	4.60±1.07	0.81±0.10	1.34±0.77	0.01±0.01	684.50±203.54
S	9.82±1.24	13.68±1.17	41.38±3.98	42.33±2.85	14.03±1.13	33.13±0.81	35.93±3.49	14.19±6.07	8.68±5.41	4.21±0.61	1.10±0.55	0.20±0.24	0.01±0.01	756.00±154.61
1 day	10.11±1.67	14.15±1.67	43.05±4.61	42.88±2.38	14.13±0.99	32.93±0.61	36.25±3.94	12.28±4.16	6.22±4.21	4.47±0.19	0.92±0.29	0.67±0.83	0.01±0.01	726.00±122.18
2 days	9.80±1.84	13.68±1.42	41.38±5.60	42.50±2.20	14.10±1.10	33.15±1.03	35.78±3.86	12.53±3.71	5.00±2.96	5.40±0.33	1.30±0.41	0.83±0.64	0.01±0.01	722.00±122.25
3 days	9.30±1.57	12.75±1.10	38.33±4.33	41.50±2.23	13.83±1.07	33.35±0.88	35.33±3.70	13.56±4.60	6.02±3.49	5.20±0.25	0.90±0.24	1.45±1.05	0.01±0.01	683.50±36.21
4 days	9.84±2.33	13.43±2.05	40.85±7.80	41.83±2.09	13.83±1.04	33.05±1.30	36.00±4.37	13.85±1.83	6.58±1.65	5.67±0.50	0.98±0.35	0.62±0.32	0.01±0.01	832.50±116.29
7 days	9.64±1.75	13.18±1.36	40.38±5.67	42.13±2.54	13.83±1.12	32.75±1.41	35.85±3.80	13.75±3.14	7.15±2.69	5.43±0.72	0.82±0.34	0.35±0.22	0.01±0.01	739.25±120.61
10 days	9.99±2.19	13.73±2.00	41.45±7.35	41.75±2.28	13.88±0.96	33.25±1.17	36.60±4.07	12.18±1.75	5.25±0.72	5.70±1.11	0.78±0.21	0.44±0.18	0.01±0.01	722.75±157.84
14 days	9.41±2.51	12.80±2.29	38.58±8.66	41.33±2.02	13.83±1.06	33.43±1.61	36.08±4.51	10.13±1.58	3.16±0.72	5.44±0.69	0.79±0.21	0.73±0.36	0.01±0.01	683.00±184.61
18 days	10.07±1.99	13.80±1.13	41.50±5.98	41.48±2.27	13.85±1.07	33.38±1.25	36.65±4.05	10.32±0.99	3.04±0.71	5.45±0.54	0.71±0.36	1.12±0.61	0.01±0.01	691.50±132.68
21 days	9.56±1.97	13.08±1.97	38.75±5.97	40.85±2.26	13.83±0.99	33.83±1.01	36.18±4.21	11.73±0.96	3.54±1.06	5.96±0.84	0.55±0.42	1.68±0.69	0.01±0.01	706.50±183.84
24 days	9.39±1.85	12.80±1.85	37.85±5.04	40.65±2.38	13.78±0.96	33.85±0.96	36.13±4.46	12.98±2.92	4.76±2.38	5.70±0.55	0.63±0.19	1.88±0.38	0.01±0.01	653.50±97.56

Abbreviations: Be S, two days before surgery; S, immediately before surgery; Ba, basophil; Eo, eosinophil; HCT, hematocrit; HGB, hemoglobin; Ly, lymphocyte; MCH, mean corpuscular volume; MCHC, mean corpuscular volume concentration; MCV, mean corpuscular volume; Mo, monocyte; Ne, neutrophil; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

Hematological parameters

Table 2 shows the postoperative hematological parameter values. Compared to the preoperative baseline total WBC count ($[10.01±1.72]×10^3$ cells/μl), the postoperative total WBC count was slightly elevated from day 1 ($[12.28±4.16]×10^3$ cells/μl) to day 7 ($[13.75±3.14]×10^3$ cells/μl) but returned to normal by day 14 ($[10.13±1.58]×10^3$ cells/μl). Compared to the preoperative baseline neutrophil count ($[3.26±1.20]×10^3$ cells/μl), the postoperative neutrophil count was slightly elevated from day 1 ($[6.22±4.21]×10^3$ cells/μl) to day 7 ($[7.15±2.69]×10^3$ cells/μl) but returned to normal by day 14 ($[3.16±0.72]×10^3$ cells/μl). Relative to the preoperative baseline lymphocyte count ($[3.26±1.07]×10^3$ cells/μl), the intraoperative lymphocyte count was suppressed ($[4.21±0.61]×10^3$ cells/μl), and the postoperative lymphocyte count was slightly suppressed on postoperative day 1 ($[4.47±0.19]×10^3$ cells/μl) but returned to normal thereafter. There were no noticeable trends for other parameters, such as monocyte counts, eosinophil counts, and basophil counts.

Serum chemistry parameters

Changes in serum chemistry parameters are summarized in Table 3. Compared to baseline preoperative AST levels (94.00±11.75 U/l), postoperative AST levels were slightly elevated from day 1 (249.75±96.60 U/l) to day 4 (192.25±95.55 U/l) but stabilized by day 7 (137.75±41.02 U/l). Compared to baseline preoperative LDH levels (1355.25±107.93 IU/l), postoperative LDH levels were slightly elevated from day 1 (2157.00±661.49 IU/l) to day 14 (1706.50±170.94 IU/l). Compared to baseline preoperative BUN levels (13.70±3.47 mg/dl), postoperative BUN levels were slightly elevated from day 1 (23.78±8.30 mg/dl) to day 3 (20.50±5.29 mg/dl) but stabilized by day 7 (12.28±1.92 mg/dl). Compared to baseline preoperative CK levels (376.50±538.36 IU/l), postoperative CK levels rapidly increased until day 2 (3109.75±3714.63 IU/l) but decreased after day 7 (133.75±70.80 IU/l). Compared to the baseline preoperative TG levels (10.75±8.26 g/dl), postoperative TG levels were suppressed on day 1 (6.00±0.82 g/dl) but

Table 3. Average values for serum chemistry parameters in Korean native cattle over the first postoperative month (mean±standard deviation)

	ALB (g/dl)	TG (g/dl)	GLU (mg/dl)	CK (IU/L)	CRE (mg/dl)	GGTP (U/L)	AST (U/L)	LDH (IU/L)	T-BIL (mg/dl)	T-PRO (g/dl)	BUN (mg/dl)
Be S	4.90±0.41	10.75±8.26	87.50±22.13	376.50±538.36	1.88±0.33	24.50±6.76	94.00±11.75	1355.25±107.93	0.18±0.04	21.38±1.80	13.70±3.47
S	5.03±0.43	8.50±2.65	74.75±12.97	159.00±80.69	1.70±0.08	24.00±7.26	97.25±30.02	1211.25±154.11	0.22±0.20	21.05±1.44	12.15±2.24
1 days	4.60±0.20	6.00±0.82	128.5±40.70	2517.00±2501.80	1.93±0.21	21.50±7.68	249.75±96.60	2157.00±661.49	0.43±0.13	20.55±0.66	23.78±8.30
2 days	5.28±0.50	10.75±6.65	99.25±19.00	3109.75±3714.63	2.20±0.29	24.75±8.18	274.75±153.27	2287.25±864.70	0.31±0.25	22.00±1.73	24.80±10.74
3 days	4.43±0.21	7.25±5.44	80.50±14.06	1730.25±972.21	2.05±0.29	26.50±9.06	219.00±100.77	1784.75±486.30	0.16±0.14	20.15±1.17	20.50±5.29
4 days	4.75±0.21	8.25±3.59	79.25±6.70	760.50±972.21	2.08±0.29	27.00±9.06	192.25±95.55	1656.25±488.81	0.28±0.14	20.15±0.82	17.13±4.49
7 days	5.03±0.13	12.25±5.91	78.50±2.38	133.75±70.80	1.88±0.38	29.00±11.92	137.75±41.02	1603.25±406.66	0.34±0.37	22.63±0.97	12.28±1.92
10 days	5.05±0.10	8.75±4.86	74.75±2.50	105.75±37.56	2.13±0.30	32.25±11.24	114.50±24.85	1494.50±310.30	0.17±0.16	26.45±0.90	13.68±2.57
14 days	5.78±0.39	12.00±6.00	65.00±9.59	140.25±29.90	2.45±0.40	35.50±7.94	135.25±22.40	1706.50±170.94	0.29±0.17	31.58±1.84	15.15±2.56
18 days	5.48±0.22	12.00±2.71	83.50±5.80	115.25±32.07	2.40±0.29	32.75±6.85	132.00±27.31	1661.50±118.04	0.19±0.11	30.68±1.30	13.18±1.80
21 days	5.18±0.39	15.00±6.68	82.75±6.99	117.25±40.62	2.23±0.36	30.50±5.97	112.75±23.67	1568.75±85.71	0.17±0.11	28.98±2.39	13.53±2.99
24 days	4.85±0.19	18.50±8.89	73.75±6.08	101.25±24.81	2.25±0.19	27.50±3.42	96.00±16.27	1482.25±201.74	0.10±0.08	27.30±0.82	14.58±3.53
28 days	5.15±0.24	21.75±7.18	82.75±8.34	166.00±93.40	2.20±0.12	29.75±4.57	101.50±13.18	1586.00±240.30	0.09±0.07	28.95±1.71	13.98±2.57

Abbreviations: Be S, two days before surgery; S, immediately before surgery; ALB, albumin; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatinine kinase; CRE, creatinine; GGTP, gamma-glutamyltranspeptidase; GLU, glucose; LDH, lactate dehydrogenase; T-BIL, total bilirubin; T-PRO, total protein; TG, triglyceride.

steadily increased until day 28 (21.75±7.18 g/dl). Compared to the baseline preoperative GLU levels (87.50±22.13 mg/dl), postoperative GLU levels were elevated on day (128.5±40.70 mg/dl) but decreased until day 3 (80.50±14.06 mg/dl). Compared to the baseline preoperative T-BIL levels (0.18±0.04 mg/dl), the postoperative T-BIL levels showed sudden elevations on days 1 (0.43±0.13 mg/dl), 4 (0.28±0.14 mg/dl), and 14 (0.29±0.17 mg/dl) but otherwise tended to decrease. Compared to the baseline preoperative GGTP levels (24.50±6.76 U/l), postoperative GGTP levels were suppressed on day 1 (21.50±7.68 U/l) but increased from day 2 (24.75±8.18 U/l) to day 28 (29.75±4.57 U/l). ALB values showed considerable variation during the experimental period, but compared to baseline preoperative ALB and CRE levels (4.90±0.41 g/dl and 1.88±0.33 mg/dl, respectively), postoperative ALB and CRE levels were elevated on days 2 (5.28±0.50 g/dl and 2.20±0.29 mg/dl, respectively) and 14 (5.78±0.39 g/dl and 2.45±0.40 mg/dl, respectively).

Rumen fluid pH parameters

Changes in rumen pH parameters are showed in Fig. 1. Compared to the rumen fluid pH values observed 2 days before the surgery (6.91±0.32), rumen fluid pH values immediately before the surgery were slightly elevated (7.66±0.83), and postoperative rumen fluid pH values were slightly elevated on day 1 (7.51±0.32) but

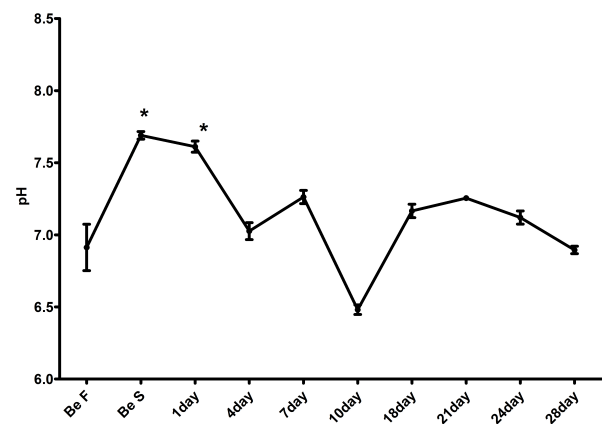


Fig. 1. Average rumen fluid pH in Korean native cattle over the first postoperative month (mean±standard error). Abbreviations: Be F, two days before surgery; Be S, before surgery; 1 day to 28 days, 1 to 28 days after surgery. *P<0.05.

decreased from day 4 (7.02 ± 0.20).

DISCUSSION

Rumen cannulation is used not only for nutritional and microbiological research and clinical diagnosis in ruminants (Leek, 1976; Perumbakkam and Craig, 2012; Terré et al, 2013) but also for transfaunation of rumen fluids (DePeters and George, 2014). Once in place, rumen cannulas allow convenient collection of representative rumen component samples (Thyfault et al, 1975). However, the various effects of surgery on the rumen environment and physical parameters of cattle must be evaluated in order to properly assess the suitability of rumen cannulation for transfaunation and research applications.

In this study, we evaluated the effects of surgery on physiological parameters and rumen fluid pH. We found that cannulation surgery had no significant effects on RBC counts or morphologies and that there was no detectable inflammation or infection of the surgical site over a 1-month follow-up period. In contrast, serum chemistry results showed changes in the activity levels of liver enzymes, including AST and CK, from postoperative days 1 to 4. These results might have been influenced by intraoperative muscle damage. Most serum chemistry parameters showed a similar pattern wherein values were increased in the first 1~2 postoperative days and decreased thereafter. The measured TG and total protein values were persistently slightly increased after surgery, but the postoperative values were not significantly different from the preoperative values.

Generally, rumen fluid pH is affected by feeding time, composition of the total mixed ration, health status, and rumen function (Enemark et al, 2003; Kristensen et al, 2010; Jiang et al, 2017). In a previous study, rumen pH values decreased from 2~8 h after feeding, and the pH values of rumen samples taken in the morning were higher than those of samples taken in the afternoon (Enemark et al, 2003; Duffield et al, 2004). The previous studies showed that the sample collection method could affect rumen pH values and that the pH values of rumen samples collected via an or-

ogastric tube were slightly higher than those of samples collected via rumen cannulation because of saliva contamination in the orogastric tube (Duffield et al, 2004; Terré et al, 2013). Additionally, rumen pH values could be affected by the sample collection site in the rumen area. A previous study reported that an oro-ruminal probe is a good method for collecting rumen samples in the central and cranial-ventral sites (Duffield et al, 2004). In this study, we collected the rumen samples via an orogastric tube between 9:00 AM and 10:00 AM each day before feeding in order to avoid false positive subacute ruminal acidosis diagnoses, which can occur following food intake. We found that rumen fluid pH values measured immediately before the surgery were slightly increased relative to those measured 2 days before the surgery but that rumen fluid pH values recovered to baseline levels by postoperative day 4. However, the postoperative values were not significantly greater than the preoperative baseline values. These results suggest that the influence of surgery on ruminal fluid pH changes thought be minor because the apparent postoperative elevation of pH values might be an effect of preoperative fasting.

Postoperative physical examinations and observations of drinking and eating behaviors did not reveal any clinical signs suggestive of inflammation or infection. Although complications of rumen cannulation surgery and local pathological changes were not observed in this study, a previous study described ruminal fluid leakage, hematoma, and abscess formation at the surgical site (Leek, 1976; Grovum, 1989; Strabel et al, 2007; Durmic et al, 2015; Rafee et al, 2015). One major concern in cannulation surgery is the size of the opening in the abdominal wall. If the opening is too much wider than the cannula, then the cannula could fall out or fall into the rumen (Laflin and Gnad, 2008; DePeters and George, 2014; Rafee et al, 2015). Stress can delay wound healing between the rumen wall and the abdominal wall by limiting the inflammatory response, which can also increase the risk of infection and surgical complications. Surgical procedures should therefore always be performed in a clean and quiet environment to reduce stress and the risk of infection. Cannulated cattle should be carefully monitored, and the surgical site should be

kept clean in order to avoid insect-borne pathogen transmission or fluid leakage.

This is the first study to examine the effects of rumen cannulation on hematological parameters and rumen pH in Hanwoo cattle. Our results suggest that rumen cannulation has no significant changes on RBC counts but changes in the activity levels of liver enzymes, such as AST and CK were observed in serum chemistry results from postoperative days 1 to 4. Additionally There were not significant changes in pH values for at least 1 month following surgery.

ACKNOWLEDGEMENTS

This study was supported by the 2016~2018 RDA Fellowship Program of the National Institute of Animal Science, Rural Development Administration and it was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Development of manual using immunological and biological indicator for cattle disease prevention, PJ01197806)”, Rural Development Administration, Republic of Korea.

Statement of Animal Rights

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. All animal experiments were approved and performed under the guidelines of the Animal Care and Experimentation Committee (No. 2016-881) of the National Institute of Animal Science, Rural Development Administration, Republic of Korea.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Alonso A. 1979. Diagnostic analysis of rumen fluid *Veterinary Clinics of North America: Large Animal Practice* 1: 363-376
- Artegoitia VM, Foote AP, Lewis RM and Freetly HC. 2017. Rumen fluid metabolomics analysis associated with feed efficiency on crossbred steers *Scientific Reports* 7: 2864
- DePeters E. and George L. 2014. Rumen transfaunation, *Immunology Letters* 162: 69-76
- Duffield T, Plaizier J, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J and McBride B. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. *Journal of Dairy Science* 87: 59-66
- Durmic Z, McGrath P, Wilmot M, Adams N, Tan T, Callahan L. and Mayberry C. 2015. Surgical and postoperative events during permanent fistulation of sheep rumen by the Schalk and Amadon method. *Australian Veterinary Journal* 93: 234-239
- Enemark J, Peters G and Jørgensen R. 2003. Continuous monitoring of rumen pH—a case study with cattle. *Journal of Veterinary Medicine* 50: 62-66
- Grovum W. 1989. An improved rumen cannulation technique to minimize leakage. *Acta Veterinaria Scandinavica. supplementum* 86: 225-228
- Jiang F, Lin X, Yan Z, Hu Z, Liu G, Sun Y, Liu X. and Wang Z. 2017. Effect of dietary roughage level on chewing activity, ruminal pH, and saliva secretion in lactating Holstein cows. *Journal of Dairy Science* 100: 2660-2671
- Kiro RP. 2017. Assessment of the rumen fluid of a bovine patient. *Journal of Dairy and Veterinary Sciences* 2: 555588
- Kleen J, Hooijer G, Rehage J and Noordhuizen J. 2004. Rumenocentesis (rumen puncture): a viable instrument in herd health diagnosis. *Deutsche Tierärztliche Wochenschrift* 111: 458-462
- Kristensen NB, Engbæk M, Vestergaard M and Harmon D. 2010. Ruminal cannulation technique in young Holstein calves: Effects of cannulation on feed intake, body weight gain, and ruminal development at six weeks of age. *Journal of Dairy Science* 93: 737-742
- Laffin SL and Gnad DP. 2008. Rumen cannulation: procedure and use of a cannulated bovine. *Veterinary Clinics of North America: Food Animal Practice* 24: 335-340
- Leek B. 1976. A simple and effective ruminal cannulation technique for sheep [proceedings]. *The Journal of Physiology* 263: 233P
- Noel SJ, Attwood GT, Rakonjac J, Moon CD, Waghorn GC Janssen PH. 2017. Seasonal changes in the digesta-adherent rumen bacterial communities of dairy cattle grazing pasture. *PloS One* 12: e0173819
- Perumbakkam S and Craig AM. 2012. Biochemical and microbial analysis of ovine rumen fluid incubated with 1, 3, 5-tri-

- nitro-1, 3, 5-triazacyclohexane (RDX). *Current Microbiology* 65: 195-201
- Rafee MA, Sinha SK and Saxena AC. 2015. Fistulation and cannulation of the rumen in buffaloes: comparison of two methods. *International Journal of Veterinary Health Science & Research* 3: 64-65
- Ramos-Morales E, Arco-Pérez A, Martín-García A, Yáñez-Ruiz D, Frutos P, Hervás G. 2014. Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats. *Animal Feed Science and Technology* 198: 57-66
- Ribeiro GO, Oss DB, He Z, Gruninger RJ, Elekwachi C, Forster RJ, Yang W, Beauchemin KA, McAllister TA. 2017. Repeated inoculation of cattle rumen with bison rumen contents alters the rumen microbiome and improves nitrogen digestibility in cattle. *Scientific Reports* 7: 1276
- Sato S. 2016. Pathophysiological evaluation of subacute ruminal acidosis (SARA) by continuous ruminal pH monitoring. *Animal Science Journal* 87: 168-177
- Strabel D, Ewy A, Kaufmann T, Steiner A and Kirchhofer M. 2007. Rumenocentesis: a suitable technique for analysis of rumen juice pH in cattle?. *Schweizer Archiv für Tierheilkunde* 149: 301-306
- Terré M, Castells L, Fàbregas F and Bach A. 2013. Comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. *Journal of Dairy Science* 96: 5290-5294
- Thyfault HA, Leffel E and Der Huang M. 1975. Simplified method for producing permanent ruminal fistulae. *Journal of Dairy Science* 58: 1899-1901
- Yilkal M, Murali MB, Ramaswamy V and Mulat A. 2016. Analysis of rumen fluid in apparently healthy slaughtered cattle at Gondar Elfora abattoir. 2016. Analysis of rumen fluid in apparently healthy slaughtered cattle at Gondar Elfora abattoir. *International Journal of Veterinary Sciences and Animal Husbandry* 1: 37-42