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ANIMAL PRODUCTION SCIENCE

Induction of hypocalcaemia and evaluation of reticuloruminal motility using a three-axis accelerometer

Woojae Choi[A], Younghye Ro[A], Dohee Kim[A], Leegon Hong[A] and Danil Kim[A,B,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Danil Kim
Department of Farm Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea
Email: danilkim@snu.ac.kr

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Andrew Fisher

ABSTRACT

Context. Reticuloruminal motility, which is continuous and regular, is essential for digestive activity, but some functional abnormalities can appear in cattle with several metabolic disorders. Particularly in periparturient dairy cows, decreased blood calcium concentrations induce deterioration of rumen motility as well as the risk of reproductive disorders. **Aims.** This study aimed to evaluate reticuloruminal motility using a bolus-type biosensor incorporated with a three-axis accelerometer (3XA) following induction of hypocalcaemia by infusion of ethylenediamine–tetraacetic acid disodium salt dihydrate (Na_2EDTA) solution. **Methods.** In the 2 × 2 crossover experiment, six non-pregnant and non-lactating cows were assigned to each of the treatment (TRE) and control (CON) groups and infused for 1 h with 13% Na_2EDTA solution and physiological saline respectively. The cylindrical biosensor was fed and placed in the reticulum before the experiment, and the three acceleration values of each cow were recorded and transmitted wirelessly. Considering the device shape, the reticuloruminal motility was represented as the vector value (V_2) calculated with each change in *X*- and *Z*-axis acceleration over time. **Key results.** Plasma calcium concentrations were measured to confirm hypocalcaemia, and the average was significantly decreased to 1.23 mmol/L at 1 h in TRE. The mean V_2 value was significantly decreased in TRE compared with CON, from 1 to 2 h after Na_2EDTA infusion. **Conclusion.** 3XA was able to detect a change in reticuloruminal motility caused by hypocalcaemia. The use of 3XA in cattle will allow for rapid treatment of hypocalcaemia or other metabolic disorders that reduce productivity. **Implications.** The 3XA inserted into the reticulum of a dairy cow detected a decrease in reticuloruminal motility wirelessly caused by induced hypocalcaemia.

Keywords: dairy cows, detection of periparturient disease, calcium, forestomach motility in cattle, induced hypocalcaemia, remote sensing, smart farming, three-axis accelerometer.

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Introduction

Rumen contraction is a unique physiological motility observed in ruminants to mix feed materials and to facilitate microbial fermentation (Foster 2017). It can be evaluated in the left paralumbar fossa via auscultation and palpation, with a rate of two to four contractions per 2 min in healthy individuals. The contraction is centrally coordinated by the vagus nerve, with regular stimulations of reticuloruminal motility, and it can be reduced when there are acidic ruminal contents, pain from illness, or a stressful environment (Ivany *et al.* 2002; Odongo *et al.* 2006; Bedford *et al.* 2020).

Calcium is an essential factor that excites the smooth muscles in the gastrointestinal tract including the forestomach in ruminants (Armstrong and Cota 1999; Perrino 2016). Decreased concentrations of extracellular blood calcium are related to forestomach hypomotility and inappetence in ruminants (Jørgensen *et al.* 1998; Goff *et al.* 2020). Particularly in dairy cows after parturition, the concentration of blood calcium decreases within 1 day after calving due to milk production and consequent demand, and the concentration cannot return to the normal range when there is not enough capacity for reabsorption or mobilisation (Jørgensen *et al.* 1998; Martinez *et al.* 2014).

In clinical hypocalcaemia, the lack of ruminal motility can induce metabolic diseases such as ketosis, and the risks of abomasal displacement and retention of placenta increase (LeBlanc *et al.* 2005; Seifi *et al.* 2011; Rodríguez *et al.* 2017). Even though there are no clinical signs, cows with decreased calcium concentrations are vulnerable to additional infections and show reduced performance in further reproduction (Venjakob *et al.* 2018).

With the recent development of information and communication technology, quantitative and continuous measurement of physiological parameters has been enabled wirelessly. Additionally, in the cattle industry, various physiological changes and behaviours can be effectively monitored using various types of biosensors (AlZahal *et al.* 2011; Stevenson *et al.* 2014; Wolfger *et al.* 2015; Neethirajan 2017). Biosensors have been applied to measure vital signs and physical activities such as body temperature, respiratory rate, lameness and exercise, and to evaluate productivity indicators such as ovarian cycles and delivery time. Among them, the implanted electrodes recorded the contraction patterns of the smooth muscle to analyse the myoelectrical activities of the reticulo-rumen in sheep, and they enabled wireless electromyography (EMG) telemetry registration (Wierzbicka *et al.* 2021). In contrast, a bolus-type accelerometer in the reticulum and a force transducer on the ruminal sac could detect both the frequency and amplitude of rumen motility accurately and effectively (Arai *et al.* 2019; Hamilton *et al.* 2019; Choi *et al.* 2020).

The present study aimed to monitor rumen motility using three-axis accelerometer (3XA) in a case of hypocalcaemia in dairy cows. For this purpose, hypocalcaemia has been experimentally induced by infusion of ethylenediamine–tetraacetic acid disodium salt dihydrate (Na_2EDTA), and confirmed by evaluation of the blood calcium concentration.

Materials and methods

Animals and ethical approval

Six non-lactating, non-pregnant Holstein cows with a mean age of 79 ± 18 (s.e.m.) months were used in the experiments. The cows were housed together in the barn with headlock feeders and allowed to access water without limitation. The same amounts of grass hay and concentrated feed were supplied for each cow twice a day. All protocols for animal experiments were validated by the Institutional Animal Care Use Committee of Seoul National University (ethical approval number SNU-190922-3, date of approval 25 November 2019).

Experimental design

The bolus-type biosensor, which incorporated a 3XA and a temperature sensor (uLikeKorea Co., Inc.), was prepared (Choi *et al.* 2020). At least 1 month before the experiments, the bolus was inserted by oral administration and settled in the reticulum.

A 2 × 2 crossover experiment was designed, and the cows were assigned to either the treatment (TRE) or control (CON) group. For the first treatment, three cows were assigned to the TRE group, and the other three cows were assigned to the CON group. For the second treatment after 1 week, the cows belonging to the TRE and the CON switched groups and received the opposite treatment. One day before each treatment, intravenous catheters (EQUIVET Hiflow long-term IV catheter 14 G × 5.25 inch, KRUUSE) were inserted in one side of the jugular vein and fixed with skin sutures, and 1 mL of heparinised saline was flushed into the catheter to prevent blood clotting. In the TRE, 13% Na_2EDTA (Sigma-Aldrich) solution was infused at a rate of 80 mg/kg for 1 h (total of 48 g of Na_2EDTA per cow), and the same volume of physiological saline (DAI HAN PHARM) was infused into the CON through the intravenous catheter. The total amount of the administered Na_2EDTA was calculated by taking the weight of the cows as approximately 600 kg. Both groups were administered simultaneously in both of the first and the second treatments. Following infusion of the Na_2EDTA solution and physiological saline for 1 h, all cows were released from the stanchion and had access to water.

Acceleration data of the device

The magnitude of each axis was recorded as the *X*, *Y* and *Z* values (longitudinal, horizontal, and vertical axes respectively), and each acceleration was influenced by the force of gravity. The vector magnitudes (*V*) and the amount of changes over time (V_1 and V_2) were calculated as follows:

$$V = \sqrt{X^2 + Y^2 + Z^2}$$

$$V_1 = \sqrt{(X_t - X_{t-1})^2 + (Y_t - Y_{t-1})^2 + (Z_t - Z_{t-1})^2}$$

$$V_2 = \sqrt{(X_t - X_{t-1})^2 + (Z_t - Z_{t-1})^2}$$

The maximum *V* values every 2 min were recorded and transferred using a wireless network. To evaluate reticular motility, the amount of change in the *V* values was primarily calculated as V_1. However, the magnitude of the *Y*-axis was changed most sensitively when the 3XA rotated along the longitudinal axis of the device (Choi *et al.* 2020). Unpredictable fluctuations of V_1 were observed even while reticuloruminal motility did not occur, and it was assumed that the bolus sensor itself was sometimes rotating by the flow of the rumen fluid; thus, the calculation was modified to exclude rotational movement of the biosensor, and the V_2 value was calculated to be used as the main acceleration value.

The transferred 3XA data were collected from 2 h before until 6 h after the start of the infusion. The average pre-infusion V_2 for 2 h and the averages of post-infusion V_2 at every 30 min interval for 6 h were calculated.

Blood chemistry analysis

Cows were held in a headlock for 1 h of the infusion, and blood sampling from the coccygeal vein with a heparin vacuum tube (lithium heparin tube; BD Vacutainer) was performed right before infusion (0 h) and at 1, 3, 6 and 24 h of the infusion. The collected blood samples were centrifuged at 2000*g* for 15 min at a room temperature of 18°C, and plasma concentrations of albumin (Alb), total cholesterol (tChol), total calcium (tCa) and inorganic phosphate (iP) were analysed using a biochemistry analyser (BS-400; Mindray, Shenzhen, China).

Statistical analyses

The average values of accelerations and the blood analysis results were statistically analysed with two-way repeated-measures ANOVA followed by the Holm–Sidak method for multiple comparisons among groups over time. All of the statistics were performed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA), and the *P*-value was set at 0.05.

Results

After each treatment for 1 h infusion had been completed, there were no cows lying down, and no changes in clinical signs were observed. The concentrations of blood samples collected at 0 h ranged between 3.4 and 4.3 g/dL for Alb, 70 and 98 mg/dL for tChol, 2.0 and 2.3 mmol/L for tCa, and between 1.5 and 2.5 mmol/L for iP, and there were no significant differences between CON and TRE. Furthermore, the concentrations of Alb and tChol were not significantly different between CON and TRE at 1, 3, 6 and 24 h (Fig. 1*a*, *b*). However, in TRE at 1 h, the end of the Na_2EDTA infusion, the average concentration of tCa was significantly decreased (1.23 mmol/L) compared with TRE at 0 h (2.20 mmol/L, $P < 0.001$) and CON at 1 h (2.18 mmol/L, $P < 0.001$; Fig. 1*c*). The concentration of tCa was increased at 3 h (1.89 mmol/L), and significant differences were still observed compared with TRE at 0 h and CON at 3 h ($P < 0.001$ for both). The concentration at 6 h (2.07 mmol/L) was significantly different only from that at 0 h ($P < 0.05$), and, finally, it was confirmed to be recovered to the level of pre-infusion at 24 h (2.23 mmol/L). The average concentration of iP was also decreased following Na_2EDTA infusion in TRE (Fig. 1*d*), and there were statistically significant differences at 1 h from that in TRE at 0 h and CON at 1 h

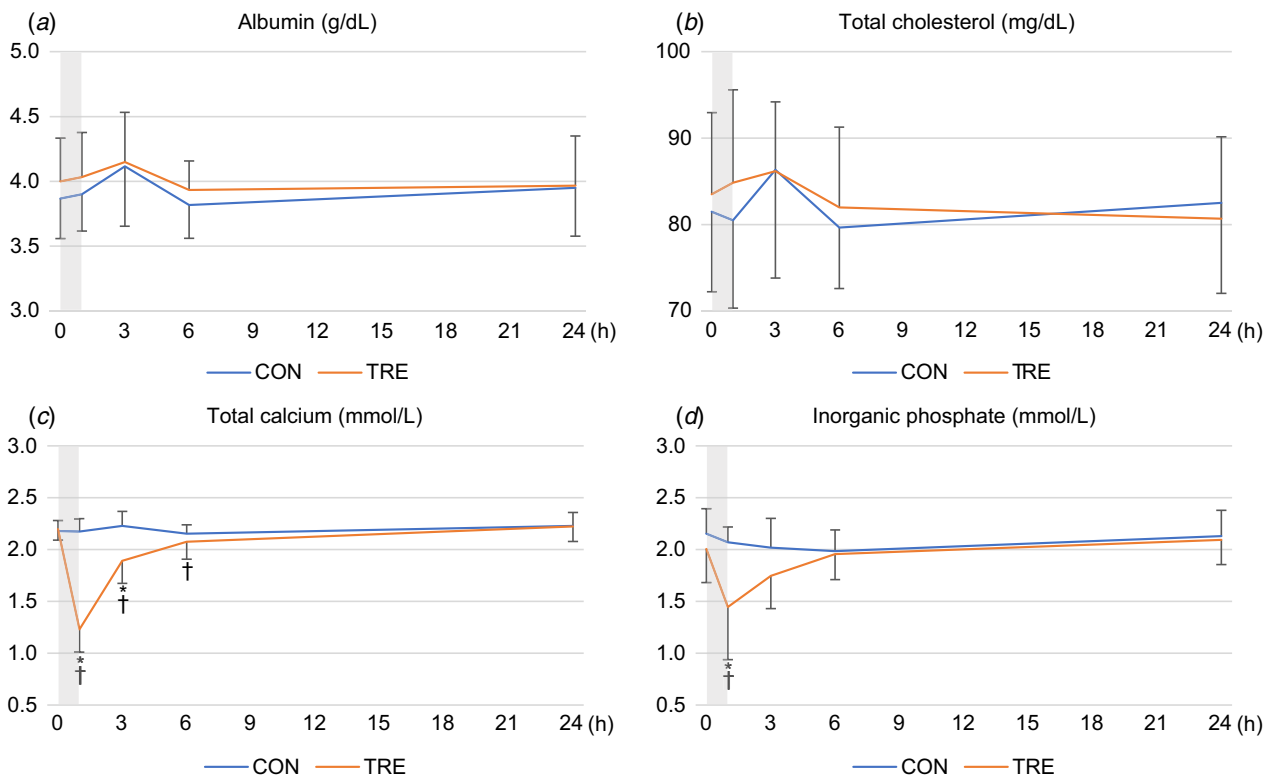

Fig. 1. The plasma concentrations of (*a*) albumin, (*b*) total cholesterol, (*c*) total calcium, and (*d*) inorganic phosphate at 0, 1, 3, 6 and 24 h after infusion. The values represent the means ± s.d. Significant differences are shown compared with the pre-infusion concentration (†$P < 0.05$) and the CON at each time point (*$P < 0.05$).

Table 1. Results of the two-way repeated-measures ANOVA for plasma concentration of total calcium and inorganic phosphate and V_2 values of the 3XA in the reticulum.

Source	d.f.	SS	MS	*F*	*P*-value
Plasma concentration of total calcium					
Time	4	2.100	0.525	101.453	<0.001
Group	1	1.091	1.091	103.277	<0.001
Time × group	4	1.950	0.488	67.934	<0.001
Plasma concentration of inorganic phosphate					
Time	4	1.008	0.252	6.317	0.002
Group	1	0.731	0.731	4.332	0.092
Time × group	4	0.729	0.182	5.432	0.004
V_2 values of the 3XA in the reticulum					
Time	12	2.27×10^5	1.90×10^4	1.755	0.077
Group	1	5.33×10^4	5.33×10^5	1.289	0.308
Time × group	12	1.85×10^5	1.54×10^5	2.258	0.020

d.f., degrees of freedom; SS, sum of squares; MS, mean squares; *F*, *F*-ratio.

($P < 0.001$ for both). The results of two-way repeated ANOVA are shown in Table 1.

As the representative results of CON and TRE, the magnitudes of three axes in 2 min intervals were obtained from one cow, and the V and V_2 of the 3XA were calculated (Fig. 2*a*). The acceleration values of each axis showed irregular fluctuations in both the CON and TRE of one cow. Among the V_2 values, a decrease in amplitude was observed from approximately 30 min in TRE and maintained for a few hours. For the average of V_2 values in each group (Fig. 2*b*), the acceleration value of TRE was decreased to 200 or less after the infusion started and maintained for approximately 1 h. In that period, the V_2 values of CON and TRE were separated from each other and showed overlapping values from approximately 2 h.

In the V_2 calculated at 30 min intervals (Fig. 3), the values for 2 h before infusion were approximately 250 in both CON and TRE, but the value of TRE was decreased to less than 200 from 0.5 h to 1.5 h after the infusion. Compared with CON, significant differences were observed from 1 to 1.5 h and from 1.5 to 2 h ($P < 0.05$ for both). Statistical significance was obtained comparing CON and TRE over time ($P < 0.05$; Table 1).

Discussion

The reduced motility of gastrointestinal tract is one of the typical symptoms that appears in most disease states in cattle, and it is an important biological sign, like body temperature and vitality. In periparturient dairy cows, changes in rumen motility can be used as an indicator of the occurrence of related disorders. In this study, therefore, we evaluated whether biosensors located in the reticulum of dairy cows can detect decreases in reticuloruminal motility and whether wirelessly transmitted data are practically helpful.

Na_2EDTA solution has been used in numerous studies of bovine hypocalcaemia (Jørgensen *et al.* 1999; Mellau *et al.* 2001), and when a chelating agent such as citrate or EDTA is administered, it diminishes ionised plasma Ca concentrations successfully in young and adult cattle (Desmecht *et al.* 1995; Martinez *et al.* 2014; Sasaki *et al.* 2014; Ro *et al.* 2020). In this study, all of the concentrations of blood samples collected at 0 h were in a normal range, but the infusion of Na_2EDTA at a total amount of 48 g for 1 h was sufficient to induce hypocalcaemia in 600 kg cows. Assuming that the largest amount of EDTA was administered at 1 h, the end of the EDTA infusion, the decreased tCa concentration reflects the chelating action of EDTA. The decreased Ca concentration recovered gradually from 1 h after the infusion (Fig. 1*c*). Parathyroid hormone (PTH) is secreted in response to decreased blood Ca concentrations, and the circulating concentration of Ca is increased by resorption from bone and reabsorption from the kidney (Kronqvist *et al.* 2011; Blaine *et al.* 2015; Ro *et al.* 2020). The concentration of iP was also decreased after Na_2EDTA infusion (Fig. 1*d*), which resulted from the PTH action on renal phosphate excretion (Mellau *et al.* 2001; Blaine *et al.* 2015). During the experimental period, the concentrations of Alb and tChol in both CON and TRE were maintained within a normal range without significant changes (Fig. 1*a*, *b*). The results indicated that the decreased tCa concentration in TRE was not due to hypoalbuminemia and was also not related to other blood parameters (e.g. cholesterol catabolism) following a short treatment time of 1 h (Onifade *et al.* 2005; Gallo *et al.* 2016).

Since the 3XA indwelling in the reticulum measures the reticuloruminal motility, which reflects the vector value according to the change of position as it is, data processing was required to show the movement of the 3XA. The V_2 value was used to better indicate the difference in reticuloruminal motility than V or V_1 by lowering the baseline of the resting period (Choi *et al.* 2020), and in this study, the V_2 value showed different accelerations between Con and TRE (Fig. 2). Among the acceleration values of V_2 with 30 min intervals (Fig. 3), the V_2 value fell below 200 from 0.5 h to 1.5 h, similar to the results after xylazine administration in a previous study (Choi *et al.* 2020). Statistical significance was evident from 1.5 h, but four of six cows started to show a decrease in V_2 at 0.5 h, and five of six cows showed a large decrease at 1 h compared with 0 h. Since the influx of extracellular Ca is important in reticuloruminal contractions (Perrino 2016), the decrease can be immediately reflected. However, individual abilities to mobilise Ca in the body were not considered in the present study.

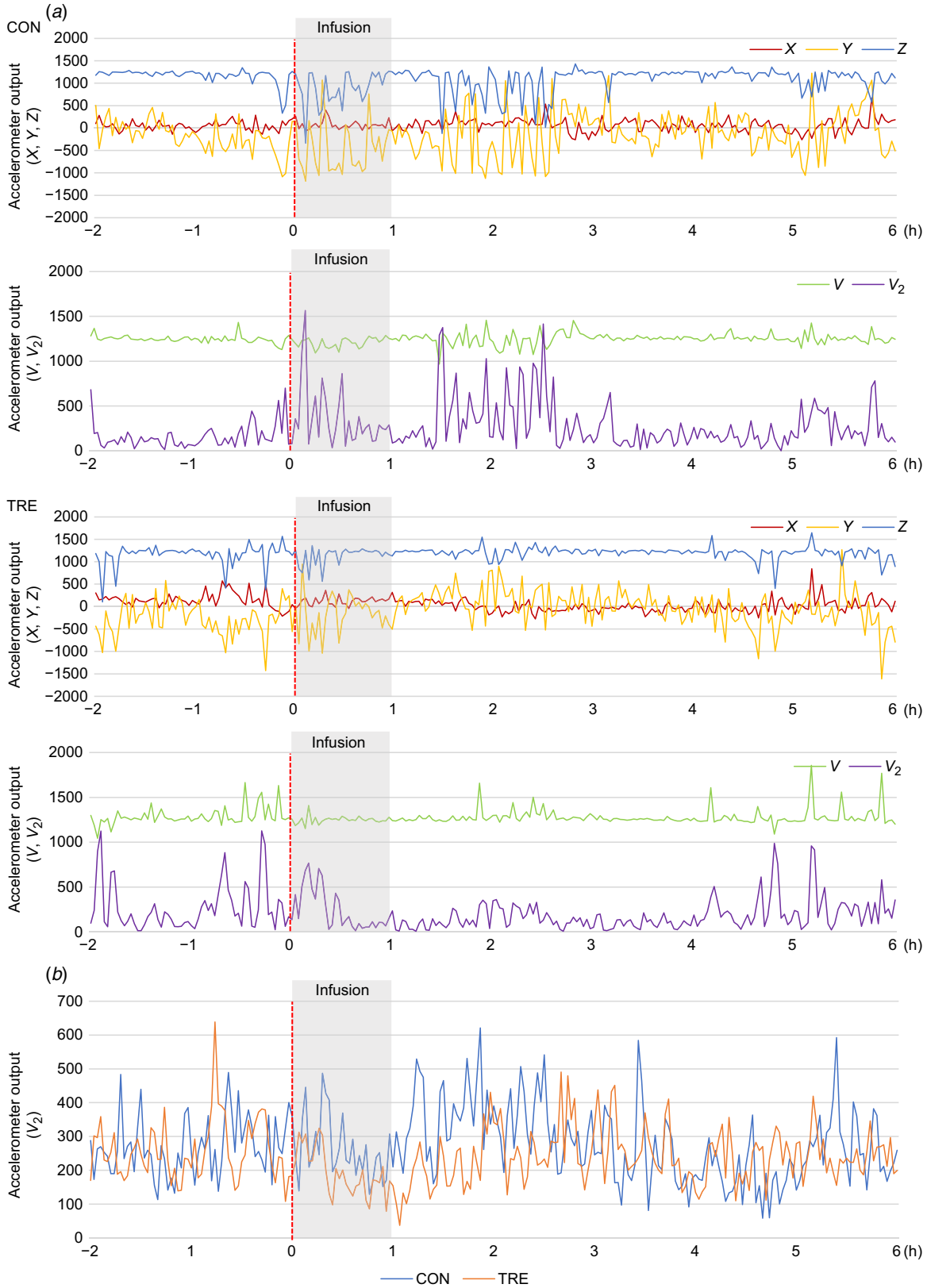

Fig. 2. The accelerometer output in 2 min intervals. The values of the three axes (*X*, *Y* and *Z*) were recorded, and the amount of vector (*V*) and its changes over time (V_2) were calculated. (*a*) A representative figure of one cow is shown, and (*b*) the mean V_2 values of six cows are represented in 2 min intervals.

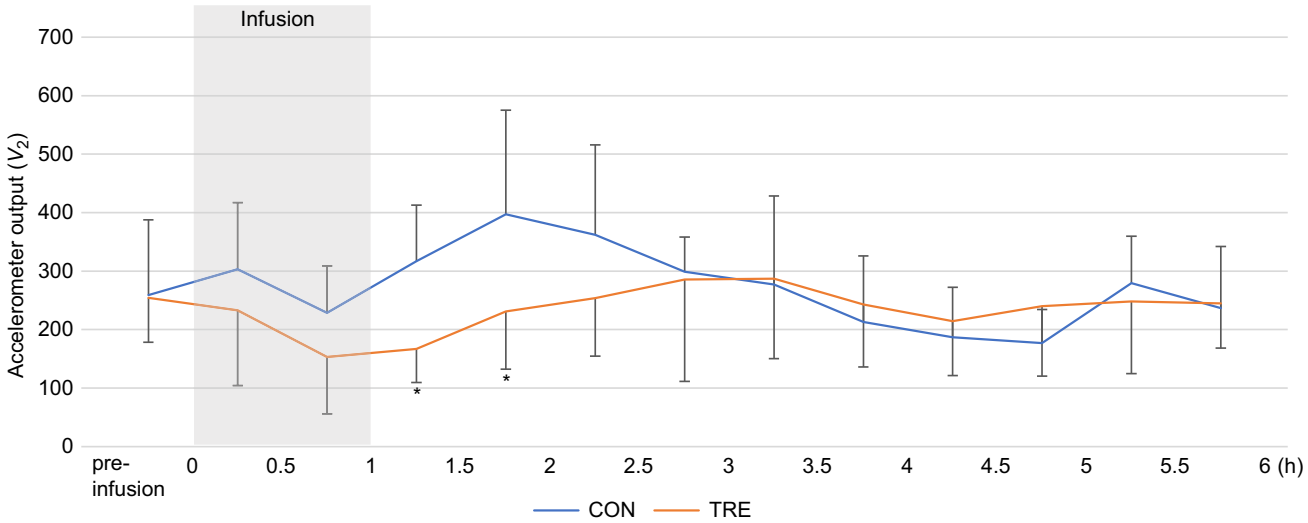

Fig. 3. The averages of V_2 values in 30 min intervals. The values represent the means ± s.d. After Na_2EDTA infusion, the V_2 values were compared between CON and TRE at each time point, and significant differences are shown from 1 to 2 h (*$P < 0.05$).

There are several limitations to this study. First, the experiment was conducted to induce hypocalcaemia in a small number of cows, and they are not representative of lactating or pregnant ones. Second, the bolus-type sensor with 3XA located in the reticulum detects and records reticuloruminal motility indirectly according to the movement of reticular walls or the flow of its contents. Recently, electrodes were surgically attached to sheep to assess the EMG activity of reticuloruminal smooth muscle directly, and the transmitted data could be recorded wirelessly for ~2 months (Wierzbicka *et al.* 2021). So as to prevent a productivity decrease in cattle caused by forestomach problems, further studies to develop a non-invasive and accurate method based on these studies are necessary.

Conclusions

The use of acceleration data wirelessly transmitted by an intrareticular biosensor with 3XA allows to record the reduction of rumen motility caused by experimentally induced hypocalcaemia.

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Data availability. The data that support this study cannot be publicly shared due to ethical or privacy reasons and may be shared upon reasonable request to the corresponding author if appropriate.

Conflicts of interest. The author declares no conflicts of interest.

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Author affiliations

[A]Department of Farm Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea.

[B]Farm Animal Clinical Training and Research Center, Institutes of Green-Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea.