Effects of Intraruminal Administration of Lactic Acid on Mineral Concentrations in Rumen Fluid of Steers

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Abstract The pH of rumen fluid was manipulated by intraruminal administration of lactic acid, acetic acid and hydrochloric acid (1 liter of 2 mol solution) and changes in soluble Ca, Mg, P, Na and K were investigated at roughage feeding period in steers fitted with rumen fistula. Lactic acid, ammonia nitrogen and VFA content in the rumen fluid as well as lactic acid level of the blood serum were determined with the infusion of 4 mol lactic acid solution. In the rumen fluid the lowest pH was observed 30 min after the acid infusion; 4.4 with hydrochloric acid, 5.9 with lactic acid and 6.2 with acetic acid. Concomitantly, when expressed in concentration, soluble Ca and Mg were almost doubled, P was slightly increased and Na and K were remained unchanged. There were highly significant correlations between pH in the rumen fluid and concentrations of Ca and Mg. The lactic acid concentration of rumen fluid, which was less than 1 mg/100 ml in steers fed the roughage, increased to about 12 mg/100 ml 30 min after the lactic acid infusion and rapidly decreased thereafter. The ammonia-nitrogen content of the rumen fluid increased after feeding and showed further increase after lactic acid infusion. The latter treatment caused a decrease of acetic acid, increases of propionic and butyric acids in the fluid, and an elevation of lactic acid level in the blood serum. The increases of soluble Ca, Mg and P were also evident when acids were added *in vitro* fermentation trials which were carried out to analyze the results of *in vivo* tests. The concentration of Na and K was again unchanged by the addition of acids *in vitro*. The results as a whole favor a view that lactic acid produced in the rumen might increase availability of some important minerals in the digesta and thereby participate mineral metabolism of the ruminants.

The pattern of rumen fermentation with ruminants fed the high grain ration is different from that fed the roughage. Several experiments¹⁻³⁾ have shown that the production of lactic acid in the rumen increased with feeding of the high grain ration. It has been known that concentrations of minerals in rumen fluid are affected by dietary levels and salivary supply of minerals according to the kinds of ration fed⁴⁻⁷⁾. In our previous reports,^{8,9)} it was suggested that the absorption of Ca, P and Mg from the digestive tract of sheep fed the high grain ration will increase in comparison with that fed the the roughage. It seems that the lactic acid produced in the rumen with the high grain ration feeding may affect the mineral concentration in rumen fluid and subsequently participate the mineral metabolism of ruminants.

The experiment reported here was conducted to clarify the relationship between the changes of mineral metabolism and increased lactic acid in rumen induced by the high grain ration feeding by the administration of lactic acid into the rumen of animals fed the roughage. Concentrations of some important minerals in rumen fluid of steers fed the roughage were determined by intraruminal infusion of lactic, acetic and hydrochloric acid solutions. Lactic acid, total VFA and ammonia-nitrogen ($\text{NH}_3\text{-N}$) in the rumen fluid and lactic acid in the blood serum

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were determined with the intraruminal administration of lactic acid. The *in vitro* fermentation trial was also conducted to analyze the results of *in vivo* tests.

Materials and Methods

Two steers (average body weight; 391 kg) fitted with permanent rumen fistula were used. Ten kg of orchardgrass hay was divided into two equal portions and was given at 9:00 and 16:00, respectively. The proximate analysis of the orchardgrass hay showed moisture 11.9, crude protein 10.4, crude fat 3.6, nitrogen free extract 38.9, crude fiber 26.2, and crude ash 9.0 percent, and the content of Ca, Mg, P, Na and K was 0.26, 0.24, 0.30, 0.02, and 2.17 percent, respectively. Water was freely available. The ration was consumed within 2.5 hours after feeding. Each quarter of 1 liter of 2 mol solutions of racemic lactic acid, acetic acid and hydrochloric acid warmed at 39°C was infused into the center part of rumen content using the long legged polyethylen funnel through rumen fistula with about 5 min intervals at 11:30, 2.5 hours after feeding. Deionized water (1 liter) was administered as the control. The administration of each acid was triplicated and the control trial was duplicated with two days intervals. About 100 ml of rumen fluid was taken at 9:00, 11:30, 12:00, 13:00, 14:00 and 16:00. The fluid was filtrated through a double cheese cloth layer and centrifuged to obtain the supernatant for determining the mineral concentration. Since no difference was found between concentrations of mineral in the supernatant of rumen fluid treated by the method of 10,000 rpm for 60 min and 3,000 rpm for 15 min, the latter method was used in this experiment. Minerals in this supernatant are regarded as the soluble ones. An aliquot of the supernatant was digested with the mixed acid (HNO_3 ; 3, H_2SO_4 ; 1, HClO_4 ; 1), made to volume with water and mineral contents were determined. The other duplicate trials were conducted with deionized water as the control and the 4 mol lactic acid solution and contents of lactic acid were determined on the blood serum and rumen fluid and that of VFA and $\text{NH}_3\text{-N}$ were also estimated on the rumen fluid. The blood samples of steers were taken from the jugular vein at the same time of rumen fluid sampling.

In the *in vitro* fermentation trial the ground orchardgrass hay passed through 1 mm screen was used as the substrate. Thirty ml of the filtrated rumen fluid taken before feeding at 9:00 AM was added to the incubation tube containing 0.5 g of the substrate. The tube was saturated with carbon dioxide and attached with a Bunsen valve and then placed at 39°C for 2.5 hours. Following this pre-incubation period, 1 ml of each acid solution was added into the tube and the incubation was further continued for 1 hour. After the incubation, the mineral content was determined by the method⁸⁾ previously reported. Sodium and K contents were determined using an atomic absorption spectrometer¹⁰⁾ (Model no. 208, Hitachi). Phosphorus content was determined colorimetrically.¹¹⁾ The pH of supernatant was estimated by a pH meter, and total VFA was determined by the steam distillation method¹²⁾ and each VFA was measured by the gas chromatography¹³⁾ (K 53 type, Hitachi). Lactic acid and $\text{NH}_3\text{-N}$ contents were estimated by the method of BARKER and SUMMERSON¹⁴⁾ and CONWAY¹⁵⁾, respectively.

Results and Discussion

Changes of the pH and mineral concentrations of rumen fluid as a function of time after administration of acids are shown in Fig. 1. Data of the control are shown as average of that obtained at the experimental periods of 2 and 4 mol acid administration. The pH in the control

trial was not affected by feeding and water infusion.

In acid trials the greatest fall of the pH was found at 30 min after administration, and the lowest pH value was observed with hydrochloric acid (4.4), followed by lactic acid (5.9) and acetic acid (6.2). Thereafter it gradually increased and returned to the initial value in 4.5 hours after dose. This experiment showed that lactic acid rather affected on the pH of rumen fluid than acetic acid, a main component of VFA, with the same molar concentration.

Calcium, Mg and K concentrations of rumen fluid increased after feeding and Na decreased,

Fig. 1 (a). Changes of the pH and mineral concentrations in the rumen fluid (Mean $\pm$ SE)

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Fig. 1 (b). Changes of the pH and mineral concentrations in the rumen fluid (Mean $\pm$ SE)

and P varied slightly. It is assumed that the increase of Ca, Mg and K are mainly affected by the dietary intake. It has been known that Na concentration in rumen fluid is more influenced by saliva than feed ingested¹⁶⁻¹⁸. It appears that the decrease of Na concentration after feeding is resulted from the low secretion of saliva according to the active fermentation in the rumen and it agrees with the observations by SAWA *et al.*⁶) and BAILEY.¹⁸⁾

After the acid infusion, the following changes of mineral concentrations were observed. Water infusion in the control trial gave no changes in concentration of each mineral through

the experimental period. Administration of acids highly increased the concentration of Ca and Mg in the rumen fluid during 30 min and levels of soluble Ca and Mg showed approximately 2 times of the initial value. Thereafter it gradually decreased and returned to the initial value at 16:00. The extent in changes of mineral concentrations were differed with the kind of acids administered and reversely corresponded with the changes of pH value. It has been observed that concentrations of Ca and Mg in rumen fluid are mainly raised by the dietary supply⁶⁾ and the amounts of these water soluble minerals in the digestive tract are affected by the pH value¹⁹⁾. It is assumed, therefore, that the acidification of rumen fluid shown as the lowered pH value by the acid administration increase the solubility of these minerals in digesta and the amount of soluble minerals in rumen fluid. The P concentration of rumen fluid was slightly varied with the acid administration. It has been reported that P concentration is generally affected by the feed intake and salivary secretion^{6,20)} with narrow variation⁸⁾. It is considered that the slight increase of P in rumen fluid in this experiment is resulted from the increased solubility of P of feed and digesta in the rumen. As it has been known that the concentration of Na in rumen fluid is mainly affected by saliva^{8,17)} and K is by saliva and feed ingested⁸⁾, it seems that less affection by acid administration on concentrations of both minerals in this experiment shows no influence of acid infusion on the secretion of saliva and the solubility of these minerals in digesta.

Table 1. The correlation coefficient between mineral concentrations and the pH in the rumen fluid with the acid administration ($n=36$)

Acid	Lactic acid	Acetic acid	Hydrochloric acid
Mineral			
Ca	-0.92**	-0.79**	-0.87**
P	-0.34*	-0.36*	0.05
Mg	-0.63**	-0.71**	-0.68**
Na	0.02	0.22	0.16
K	-0.24	0.04	-0.14

** : $P < .001$

* : $P < .05$

The coefficients of correlation between the pH value and the concentration of each mineral in rumen fluid after the acid administration are shown in Table 1. The negative correlation on Ca and Mg concentration was significantly high with all the acids administered ($P < .001$). These results suggested that concentrations of these minerals were mainly affected by the rumen pH. The correlation between P and pH value was significant with the lactic and acetic acid administration ($P < .05$), and not significant with hydrochloric acid. There was little correlation between K and Na concentration and the pH value with all the acid administered.

Changes of the pH value and the concentration of Ca and Mg are shown in Fig. 1 with the administration of 4 mol lactic acid solution. The more decrease of pH and more increase of these mineral concentrations occurred as compared with the infusion of 2 mol lactic acid. Concentration of lactic acid, $\text{NH}_3\text{-N}$, total VFA and individual VFA in rumen fluid and of lactic acid in blood serum are shown in Figs. 2 and 3. These values were also determined on the sample of the control trial at the period of 4 mol acid administration. The lactic acid concentration of rumen fluid in the control trial showed a constant level and was less than 1 mg per 100 ml, and that in the lactic acid trial markedly increased and attained to about 12 mg at 30 min after infusion. Subsequently it gradually declined to about 3 mg per 100 ml at 16:00.

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Fig. 2. Changes of the ammonia-nitrogen and lactic acid content in the rumen fluid and the level of serum lactic acid with 4 mol lactic acid administration (Mean $\pm$ SE, $n=4$)

Fig. 3. Changes of total VFA and individual VFA contents in the rumen fluid with 4 mol lactic acid administration (Mean $\pm$ SE, $n=4$)

The level of lactic acid in blood serum increased with the acid administration into the rumen and the highest value which was approximately 2 times higher than that of the control was found at 13:00. It seems that these changes are resulted from the conversion of lactic acid to other products, namely propionic acid in the rumen and the absorption of lactic acid from rumen wall^[21-24]. Level of $\text{NH}_3\text{-N}$ in the rumen fluid increased after feeding and the highest

value was found at 11:30 and it increased moreover after the acid administration. Total VFA and the ratio of individual VFA in rumen fluid remained constant in the control. The molar percent of acetic acid markedly decreased with increase of propionic and butyric acids with the acid administration. The increased ratio of propionic and butyric acid in this experiment seemed to be resulted from high conversion of lactic acid^{25,28}).

Table 2. The pH and mineral concentrations (mg/100 ml) in the rumen fluid at 1 hour after the acid administration (*In vitro* trial, mean of three determinations)

Treatment	pH	Ca	P	Mg	Na	K
Control						
(Water)	6.9	9.2	63.9	9.5	175	219
Lactic acid						
(2 mol)	5.5	10.6	68.0	10.3	171	222
(4 mol)	4.6	11.7	69.0	10.6	176	223
Acetic acid						
(2 mol)	5.4	10.2	71.6	10.3	173	222
(4 mol)	4.9	10.8	68.8	10.5	176	222
Hydrochloric acid						
(2 mol)	5.0	10.4	70.3	10.5	176	220
(4 mol)	4.2	11.7	70.5	10.8	176	223

The variations of mineral concentrations with each acid addition *in vitro* fermentation trial are shown in Table 2. Concentrations of Ca, P and Mg in rumen fluid after incubation were higher with the addition of 2 mol solution of acid compared to that of the control. Sodium and K concentrations were not influenced by the acid addition. The further decrease of the pH value and increase of concentration of Ca, P and Mg were observed when the 4 mol solution of acid was added. Changes of the pH value and mineral concentration were differed with the kind of acid added and those were similar to that found *in vivo* trials. The lowered increasing rate *in vitro* trial seems to be resulted from the limit of supplying the substrate. It seems that the increased soluble minerals observed with the acid administration are derived from the substrate and rumen fluid owing to the increased solubility of minerals without any affection by salivary supply. The change of P concentration contributed by the acid addition was smaller than that of Ca and Mg, and concentrations of Na and K were not affected by the pH value varied with the acid administration.

It is evident therefore that the lactic acid administered into the rumen of steers fed the roughage temporarily lowered the pH value of rumen fluid and subsequently increased the concentration of Ca, P and Mg of rumen fluid and further increased the lactic acid level in blood serum. It was observed in our previous experiments^{8,9}) that the absorption and urinary excretion of Ca, P and Mg in sheep fed the high grain ration was significantly higher than that of sheep fed the roughage, suggesting that the absorption of these minerals from the digestive tract would be more stimulated when sheep was fed the high grain ration. It is assumed therefore that the continual production of lactic acid in the rumen of animal fed the high grain ration would play an important role on mineral metabolism as same to the observations found with the intraruminal administration of lactic acid for animals fed the roughage.

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ルーメン液の無機物濃度に対する乳酸投与の影響

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反芻家畜に穀類を多給した時にみられる無機物代謝の変化と、ルーメン内で産生が高まる乳酸との関係を解明するため、乳酸産生の低い粗飼料給与時のルーメン内に乳酸を投与する実験を行った。オーチャードグラス乾草給与のルーメンフィステル装着牛を用い、フィステルより乳酸、酢酸および塩酸(2 モル溶液, 1 liter)をルーメン内に直接投与し、ルーメン液の無機物濃度および pH 値の変化ならびに 4 モル乳酸溶液投与時のルーメン液の乳酸、アンモニア態窒素、全 VEA 量、各 VFA モル比および血中乳酸量を経時的に測定した。また *in vitro* 試験法による各酸添加と培養液の無機物濃度との関係について *in vivo* での成績と比較した。

ルーメン液の pH 値は酸の投与により急激に低下し(酢酸; 0.9, 乳酸: 1.3, 塩酸; 2.6 pH 単位低下), 投与後 30 分で最低値を示しその後徐々に上昇して投与後 4.5 時間にほぼ投与前の値になった。ルーメン液の Ca および Mg 濃度はルーメン液 pH 値の変化と高い相関を持って変化し、酸の投与後 30 分に投与前の約 2 倍に急増し、

その後 pH 値の上昇に伴って減少し、投与後 4.5 時間にほぼ投与前の値になった。P 濃度は Ca および Mg と同様の傾向を示したが、その増減割合は小さかった。Na および K 濃度の酸投与による変化はさらに小さく、pH の変化による影響は認められなかった。4 モル乳酸液投与時の Ca および Mg 濃度は 2 モル乳酸液の場合より高い値を示した。ルーメン液の乳酸濃度は投与後、時間の経過と共に減じたが、プロピオン酸および酪酸は増加する傾向を示した。血中乳酸濃度は乳酸投与後若干増加した。*In vitro* 試験において、培養液の Ca および Mg 濃度は、酸の添加量を増加するに従って上昇し、飼料からの無機物溶出量およびルーメン液の可溶性無機物濃度が増大することを明らかに示したが、この濃度の増加割合は、*in vivo* の成績と比較して低いものであった。穀類多給時のルーメン内乳酸産生量の増加はルーメン液の主要無機物濃度および血液乳酸量の増加を介して、反芻家畜の無機物代謝に影響を与えているものと考えられた。