



Responses of Blood Glucose, Insulin, Glucagon, and Fatty Acids to Intraruminal Infusion of Propionate in Hanwoo

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ABSTRACT: This study was carried out to investigate the effects of intraruminal infusion of propionate on ruminal fermentation characteristics and blood hormones and metabolites in Hanwoo (Korean cattle) steers. Four Hanwoo steers (average body wt. 270 kg, 13 month of age) equipped with rumen cannula were infused into rumens with 0.0 M (Water, C), 0.5 M (37 g/L, T1), 1.0 M (74 g/L, T2) and 1.5 M (111 g/L, T3) of propionate for 1 hour per day and allotted by 4×4 Latin square design. On the 5th day of infusion, samples of rumen and blood were collected at 0, 60, 120, 180, and 300 min after intraruminal infusion of propionate. The concentrations of serum glucose and plasma glucagon were not affected ($p>0.05$) by intraruminal infusion of propionate. The serum insulin concentration at 60 min after infusion was significantly ($p<0.05$) higher in T3 than in C, while the concentration of non-esterified fatty acid (NEFA) at 60 and 180 min after infusion was significantly ($p<0.05$) lower in the propionate treatments than in C. Hence, intraruminal infusion of propionate stimulates the secretion of insulin, and decreases serum NEFA concentration rather than the change of serum glucose concentration. (**Key Words:** Rumen, Propionate Infusion, Hormones, Steer)

INTRODUCTION

Beef quality is mostly influenced by intramuscular fat content of meat which is a metabolic factor influencing meat tenderness, juiciness and flavor (Winger and Hagyard, 1994). Intramuscular fat synthesis in turn is mostly influenced by glucose, which is the preferred product of glycolytic fibers and the major precursor of glycogen and intramuscular fat synthesis (Hocquette et al., 1998).

However, glucose is poorly absorbed and originates mostly from hepatic gluconeogenesis, which varies with metabolizable energy intake, the pattern of ruminal

fermentation, the supply of gluconeogenic substrates as well as the hormonal status and energy requirements of animals (Drackley et al., 2001).

Energy metabolism by ruminants largely depends on volatile fatty acids (VFA) from microbial fermentation in the fore-stomachs and hindgut (Bergman, 1990). Propionate can contribute more than 32% to 73% of hepatic glucose synthesis in ruminants, and supplementation of propionate increased whole-body glucose turn over by 13% to 59% in growing steers (Seal and Parker, 1994). These responses were associated with increased serum insulin concentrations as reported by Casse et al. (1994) who found that propionate infusion at 3 days slightly reduced net hepatic glucose release and elevated insulin secretion in lactating cows. Therefore, propionate is expected to influence plasma metabolism in ruminants. The effects of propionate on hepatic gluconeogenesis can be associated with differences in insulin metabolism (Donkin et al., 1997), energy balance or glucose requirements of animals (Drackley et al., 2001). Compromised propionate production may reduce glucose production (DiCostanzo et al., 1999). Infusion of VFA into the blood have stimulated release in insulin (Horino et al.,

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1968), whereas ruminal infusions have had no effect on plasma concentrations of glucose or insulin (Stern et al., 1970)

The objective of this study was to investigate the change of the ruminal fermentation characteristics and blood hormones and metabolites following infusion of propionate directly into the rumen of Hanwoo steers.

MATERIALS AND METHODS

Animals and diet

Ruminally cannulated Hanwoo steers ($n = 4$) at the age of approximately 13 months and an average body weight (BW) of approximately 270 kg were used in this study. Animals were allotted in a 4×4 Latin square design for intraruminal infusion of propionate.

Ingredients and chemical compositions of the diet fed to experimental animal are shown in Table 1. A concentrate was formulated in the feed factory of the National Institute of Animal Science. The diet was fed as 1.5% of BW of experimental animals and contained 50% concentrate and 50% rice straw at twice per day (10:00 and 19:00) equally for each individual steer. This feeding level was determined throughout the preliminary experiment when feed intake was not decreased by intraruminal infusion of propionate, and was at 80% of National Research Council (NRC, 2001) requirements for beef cattle.

Intraruminal infusion of propionate

Four solutions of 0.0 M (deionized water; C), 0.5 M (T1), 1.0 M (T2), and 1.5 M (T3) of propionate were

continuously infused (1 L per hour) into the rumens of Hanwoo steers through the rumen cannula. Treatment solutions were prepared by adjusting the pH 7.0 with potassium hydroxide and sodium hydroxide after solving 0, 37, 74, and 111 g of propionic acid in 1 L of deionized water.

The propionate solution was infused at 3 hour after am feeding (13:00) for 1 hour per day during 5 days and the intervals to next infusion were 3 days. Solutions were infused using 4-channel peristaltic pumps (505S, Watson-Mallow Ltd., Cornwall, UK) and Tygon tubing (7.5 m×1.6 mm i.d.; Fisher Scientific Co., Pittsburgh, PA, USA).

Sample collections

Rumen and blood samples were collected at 0, 60, 120, 180, and 300 min after infusion of solution through rumen cannula and jugular vein catheter on the 5th day after infusion, respectively. A jugular vein catheter was attached on a day before collection to reduce the stress of its adhesion to the neck. Rumen fluid was collected from three different sites in the rumen and squeezed through 8 folds of cheesecloth, and pH was determined immediately after collection. Rumen fluid was stored at -70°C for determination of concentration of VFA. Blood plasma for glucagon determination was obtained by centrifugation (2,500 rpm for 20 min) the whole blood supplemented Ethylenediaminetetraacetic acid as an anticoagulant and blood serum for determination of insulin, glucose and non-esterified fatty acid (NEFA) was obtained by coagulation for 1 hour at 4°C . Blood plasma and serum were stored at -70°C until analyzed.

Analyses of diet, ruminal fluids, and blood samples

The chemical composition of concentrate and roughage was determined by AOAC (1995) for proximate analysis of crude protein, ether extract, and crude fiber and the cell wall constituents as neutral detergent fiber, and acid detergent fiber of diet were determined according to the method of Goering and Van Soest (1970).

The pH of rumen fluids was determined using a pH meter (Orion 920A, Thermo Electron Co., Washington DC, WA, USA). Concentration of VFA in rumen fluids was determined using the gas chromatography (VISTA 6000, Varian Associates Inc., Santa Clara, CA, USA) according to the method modified by Czerkawski (1976).

After pretreatment with the analysis kits (Chiron Diagnostics Co., Oberlin, OH, USA), blood hormones and metabolites were measured by the sandwich enzyme-linked immunosorbent assay reader (ELP-40, Bio-Tek Instruments, Colar Cedex, France) for serum insulin, the gamma-counter (5002 Cobra System, Packard Instrument Co., Meriden, CT, USA) for plasma glucagon, the spectrophotometer (Spectronic 601; Milton-roy Co., Ivyland, PA, USA) for

Table 1. Ingredients and chemical composition of the diets fed experimental animals

Items	Concentrate	Rice straw
Ingredients, as-fed basis (%)		
Corn	55.0	
Wheat bran	23.0	
Soybean meal	20.0	
Salt	0.7	
Limestone	0.5	
Tri-calcium phosphate	0.5	
Vitamin-mineral premix ¹	0.3	
Chemical composition ² (dry matter basis, %)		
Crude protein	20.94	5.72
Ether extract	3.59	1.15
Crude fiber	6.42	36.31
Nitrogen free extracts	62.18	44.70
Neutral detergent fiber	48.10	80.48
Acid detergent fiber	9.07	52.50

¹ Supplied per kilogram of diet : 6,000 IU vitamin A, 1,022 IU vitamin D₃, 80 mg K, 50 mg Zn, 50 mg S, 40 mg Mn, 30 mg Fe, 10 mg Cu, 0.53 mg I, 0.50 mg Co, and 0.13 mg Se.

² Analytical values.

serum NEFA, and the blood auto analyzer (Express Plus, Ciba Corning Diagnostics Corp., Irvine, CA, USA) for serum glucose, respectively.

Statistical analyses

The effect of propionate infusion into the rumen of Hanwoo steers was examined in a model that included treatment and sampling time. Data were analyzed as repeated measures using the General Linear Model procedure of SAS (1999). Duncan's Multiple Range Test was used to test the significance ($p < 0.05$) of differences among means.

RESULTS

The changes of ruminal pH by infusion of propionate into the rumen are shown in Table 2. The intraruminal infusion of propionate did not affect ($p > 0.05$) the pH values of rumen fluids and the pH values ranged from 6.68 to 7.02 at 300 min post-infusion.

The intraruminal infusion of propionate showed no effect ($p > 0.05$) on the concentrations of total VFA, acetate, isobutyrate, butyrate, isovalerate and valerate in rumen fluids, while the propionate concentrations at 60, 120, and 180 min after infusion were significantly ($p < 0.05$) higher in T2 and T3 than in C and peaked at 60 min after infusion of propionate (Table 3).

The concentration of ruminal VFAs after propionate infusion was significantly ($p < 0.05$) higher at 60 min than at 300 min for total VFA in T2 and T3, and significantly ($p < 0.05$) higher at 60 min than at 0 and 300 min for propionate in the all propionate treatments.

The ratio of acetate to propionate at 60, 120, and 180 min after infusion was significantly ($p < 0.05$) lower in T1, T2, and T3 than in C and was lowest in T3. The ratio of acetate to propionate at 300 min after infusion was significantly ($p < 0.05$) lower in T2 and T3 than in C and T1, but was not different ($p > 0.05$) between T2 and T3.

The concentrations of serum insulin and plasma

glucagon are shown in Table 4. The concentration of serum insulin was significantly ($p < 0.05$) higher in T3 than in C at 60 min after infusion of propionate, but was not different ($p > 0.05$) among treatments at 30, 90, 120, and 180 min after infusion. The concentration of serum insulin reached a plateau ($p < 0.05$) at 60 min after propionate infusion in T2 and T3, but was not affected by elapsing time after infusion in C and T1.

The concentration of plasma glucagon was not affected ($p > 0.05$) by treatment and post-infusion time of propionate even at 60 min after infusion when insulin was significantly ($p < 0.05$) affected by treatment.

The changes of concentration of glucose and NEFA in serum of Hanwoo steers by intraruminal infusion of propionate are shown in Table 5. The concentration of serum glucose was not affected ($p > 0.05$) by treatment and time after infusion. The concentration of serum NEFA was significantly ($p < 0.05$) lower in T3 than in C and T1 at 30 min after infusion and was significantly ($p < 0.05$) lower in T1, T2, and T3 than in C at 60 and 180 min after infusion. The concentration of plasma NEFA was highest ($p < 0.05$) at 300 min after propionate infusion in all treatments. As a result, intraruminal infusion of propionate stimulated the secretion of insulin and decreased serum NEFA concentration rather than produce a change in serum glucose concentration.

DISCUSSION

Volatile fatty acids are derived primarily from microbial fermentation and provide roughly 70% of the energy requirement in sheep and VFA can stimulate insulin and glucagon release in ruminants (Lee and Hossner, 2002). Particularly at higher propionate concentrations in the rumen, gluconeogenesis continues to be stimulated in the liver (Lobley et al., 2000). Although approximately 30% of propionate produced in the rumen may escape into the abomasum and omasum, propionate could be completely metabolized within the post-ruminal tissues (Lobley et al., 2000).

The present experiment showed that intraruminal infusion of propionate had no effect on the pH values in rumen fluids that ranged from 6.68 to 7.02 at 300 min post-infusion. There was no effect in rumen pH due to the levels of propionate infusion because all infusates including the water were adjusted to pH 7.0 by alkali. The intraruminal infusion of propionate increased propionate concentration and decreased acetate to propionate ratio of ruminal fluids in the present experiment. Abdul-Razsqa et al. (1988) reported that isoenergetic rations with a low ratio of acetate to propionate in ruminal fluid promoted greater fat deposition in sheep. While the ratio of sodium propionate in a roughage-based diet decreased fat deposition in sheep (Van Houtert and Leng, 1993).

Table 2. Effects of intraruminal infusion of propionate on pH value in the rumen fluids of Hanwoo steers

Item	Treatment ¹				SEM
	C	T1	T2	T3	
Time after infusion, min					
0	6.70	6.67	6.74	6.65	0.020
60	6.68	6.76	6.73	6.61	0.033
120	6.73	6.75	6.69	6.72	0.012
180	6.79	6.84	6.83	6.81	0.011
300	7.00	6.98	6.96	7.02	0.011

SEM, standard error of the mean.

¹ Propionate solutions were continuously infused 0.0 M (C), 0.5 M (T1), 1.0 M (T2), and 1.5M (T3) at 1L per hour in the rumens through the cannula.

Table 3. Effects of intraruminal infusion of propionate on volatile fatty acids concentration in the rumen fluids of Hanwoo steers

Item	Treatment ¹				SEM
	C	T1	T2	T3	
Time after infusion (min)	----- Total VFA, mM/L -----				
0	78.55	74.55	72.13 ^{AB}	78.07 ^{AB}	1.521
60	63.10	82.06	88.77 ^A	108.71 ^A	9.411
120	64.71	73.88	82.82 ^{AB}	84.89 ^{AB}	4.620
180	55.17	64.90	75.23 ^{AB}	69.07 ^{AB}	4.214
300	51.43	50.36	56.37 ^B	51.46 ^B	1.346
Time after infusion (min)	----- Acetate, mM/L -----				
0	54.01	50.61	50.35	53.92	1.008
60	43.27	44.92	47.52	51.20	1.729
120	44.63	44.20	47.12	43.29	0.819
180	38.29	40.39	45.25	37.37	1.759
300	35.78	34.51	34.84	30.11	1.262
Time after infusion (min)	----- Propionate, mM/L -----				
0	14.43	13.93 ^{BC}	12.44 ^D	14.39 ^C	0.467
60	11.60 ^c	26.00 ^{bcA}	32.26 ^{bA}	47.98 ^{aA}	7.538
120	11.73 ^c	20.87 ^{bcAB}	26.30 ^{abAB}	34.86 ^{aAB}	4.850
180	9.82 ^b	16.17 ^{abBC}	21.09 ^{aBC}	24.20 ^{aBC}	3.137
300	8.95	10.62 ^C	14.77 ^{CD}	15.93 ^C	1.659
Time after infusion (min)	----- Isobutyrate, mM/L -----				
0	1.14	1.00	0.99	1.03	0.034
60	0.88	1.09	0.94	1.00	0.045
120	0.88	0.85	0.96	0.72	0.050
180	0.75	0.84	0.93	0.82	0.037
300	0.77	0.62	0.75	0.66	0.036
Time after infusion (min)	----- Butyrate, mM/L -----				
0	7.03	7.31 ^{AB}	6.66	7.04	0.133
60	5.97	8.37 ^A	6.64	7.06	0.506
120	6.15	6.68 ^{AB}	7.03	5.03	0.437
180	5.20	6.22 ^{AB}	6.63	5.48	0.329
300	4.78	3.80 ^B	4.96	3.92	0.295
Time after infusion (min)	----- Isovalerate, mM/L -----				
0	0.79	0.70	0.69	0.69	0.024
60	0.58	0.70	0.56	0.65	0.032
120	0.57	0.53	0.58	0.45	0.030
180	0.49	0.56	0.54	0.52	0.015
300	0.54	0.37	0.46	0.37	0.041
Time after infusion (min)	----- Valerate, mM/L -----				
0	1.15	1.00	0.99	1.00	0.038
60	0.80	0.97	0.85	0.83	0.037
120	0.76	0.75	0.83	0.54	0.063
180	0.62	0.71	0.78	0.68	0.033
300	0.60	0.44	0.58	0.47	0.040
Time after infusion (min)	----- Acetate/propionate -----				
0	3.81	3.75 ^A	4.16 ^A	3.79 ^A	0.095
60	3.85 ^a	1.73 ^{bc}	1.47 ^{bcC}	1.06 ^{cd}	0.623
120	4.01 ^a	2.17 ^{bcBC}	1.81 ^{bcBC}	1.24 ^{cd}	0.599
180	4.14 ^a	2.60 ^{bb}	2.18 ^{bcBC}	1.54 ^{cc}	0.553
300	4.25 ^a	3.49 ^{aa}	2.36 ^{bb}	1.94 ^{bb}	0.527

SEM, standard error of the means.

¹ Propionate solutions were continuously infused 0.0 M (C), 0.5 M (T1), 1.0 M (T2), and 1.5 M (T3) at 1 L per hour in the rumens through the cannula.^{a,b,c} Values in the same row with different superscripts differ at p<0.05.^{A,B,C} Values in the same column with different superscripts differ at p<0.05.

Table 4. Effects of intraruminal infusion of propionate on the concentration of serum insulin and plasma glucagon in Hanwoo steers

Item	Treatment ¹				SEM
	C	T1	T2	T3	
Time after infusion (min)	----- Insulin, μ U/mL -----				
0	19.84	21.67	19.94 ^B	26.03	1.449
30	21.15	25.41	38.12 ^A	41.63	4.921
60	20.70 ^b	33.38 ^{ab}	42.43 ^{abA}	53.80 ^a	7.007
90	15.63	22.54	25.66 ^B	33.48	3.701
120	19.75	21.95	23.61 ^B	23.66	0.921
180	20.61	22.11	26.00 ^B	21.41	1.196
Time after infusion (min)	----- Glucagon, pg/mL -----				
0	41.88	48.48	45.42	44.52	1.361
30	39.37	53.84	46.45	57.98	4.109
60	40.96	54.58	54.24	56.71	3.596
90	37.24	45.15	51.57	45.74	2.943
120	39.47	44.71	60.91	52.38	4.672
180	39.97	41.15	52.80	43.55	2.908
300	40.55	41.00	49.89	38.09	2.583

SEM, standard error of the means.

¹ Propionate solutions were continuously infused 0.0 M (C), 0.5 M (T1), 1.0 M (T2), and 1.5 M (T3) at 1 L per hour in the rumens through the cannula.

^{a,b} Values in the same row with different superscripts differ at $p < 0.05$.

^{A,B} Values in the same column with different superscripts differ at $p < 0.05$.

The insulin concentration of serum was increased at 1 hour after intraruminal infusion of propionate in the present experiment. Evans et al. (1975) reported that plasma insulin concentrations peaked at 0.5 hour and 5.5 hour post feeding for cows, and were greater immediately after feeding than at 0.5 hour before feeding and 1.5 hour after feeding in sheep fed high concentrate diet. Bines and Hart (1984) reporting plasma hormones and metabolite responses to intraruminal

infusion of VFA mixtures in cattle found that insulin concentrations were less when propionate was omitted from the infusate. Moreover, Istasse et al. (1987) reported that infusion of propionate into the rumen increased insulin concentration of plasma without any change in plasma glucose concentration. In the present experiment, the intraruminal infusion of propionate increased serum insulin concentration and this result was similar to the reports of

Table 5. Effects of intraruminal infusion of propionate on the concentrations of serum glucose and plasma NEFA of Hanwoo steers

Item	Treatment ¹				SEM
	C	T1	T2	T3	
Time after infusion (min)	----- Glucose, mg/100 mL -----				
0	79.25	79.75	79.67	81.25	0.437
30	78.50	81.00	83.00	84.50	1.299
60	78.75	79.75	82.00	81.00	0.711
90	80.00	80.00	80.50	80.75	0.188
120	79.00	78.75	81.25	81.75	0.766
180	78.50	78.50	81.50	78.50	0.750
300	82.00	81.00	81.00	81.00	0.250
Time after infusion (min)	----- NEFA, μ Eq/L -----				
0	54.33 ^D	50.33 ^D	89.67 ^C	92.67 ^{AB}	11.26
30	124.00 ^{aC}	90.67 ^{aB}	83.67 ^{abC}	44.50 ^{bC}	16.31
60	167.67 ^{aBC}	54.00 ^{bD}	76.00 ^{bC}	57.00 ^{bC}	26.78
90	171.00 ^{BC}	75.00 ^C	110.33 ^B	58.00 ^C	24.98
120	124.50 ^C	70.00 ^C	122.00 ^{AB}	76.00 ^B	14.57
180	178.67 ^{aAB}	93.33 ^{bB}	71.33 ^{bC}	74.33 ^{bB}	25.23
300	223.00 ^A	218.00 ^A	189.33 ^A	175.50 ^A	11.40

NEFA, non-esterified fatty acid; SEM, standard error of the means.

¹ Propionate solutions were continuously infused 0.0 M (C), 0.5 M (T1), 1.0 M (T2), and 1.5 M (T3) at 1 L per hour in the rumens through the cannula.

^{a,b} Values in the same row with different superscripts differ at $p < 0.05$.

^{A,B,C,D} Values in the same column with different superscripts differ at $p < 0.05$.

Sano et al. (1993; 1995).

The intraruminal infusion of propionate did not affect the glucagon and glucose concentrations of blood in the present experiment. Sano et al. (1993) reported that infusion of propionate in mesenteric vein increased plasma insulin concentration, but plasma glucose concentration remained unchanged in sheep. Therefore, plasma insulin response to propionate must use different mechanisms from those affecting plasma glucose concentrations. Hence, in the present experiment, intraruminal infusion of propionate may also use a different mechanism to transport propionate into mesenteric veins.

Plasma glucagon responses to VFA are generally less than are insulin responses (De Jong, 1982). On the other hand, Sano et al. (1993; 1995) reported that infusion of propionate into femoral and mesenteric veins increased plasma glucagon concentration in sheep and indicated that the magnitude of plasma glucagon responses is influenced by the rate of propionate removal by the liver. Therefore, plasma glucagon responds to infusion of propionate that travels through the portal vein and is removed by the liver (Lobley et al., 2000). In the present experiment the intraruminal infusion of propionate decreased NEFA concentration of serum at 30, 60, and 180 min after infusion. Lemosquet et al. (1997) reported that duodenal infusion of glucose increased concentration of glucose and insulin in serum or postprandial plasma and decreased NEFA concentration in plasma.

In conclusion, the results of the present experiment indicate that intraruminal infusion of propionate (111 g/d) significantly increased serum insulin, while no change was observed on the concentration of serum glucose and plasma glucagon. This may indicate that propionate produced in the rumen and absorbed into the circulatory system stimulates insulin secretion and the concentration of insulin and NEFA in serum is in a negative relationship.

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