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Full Length Research Paper

Effects of acetic, propionic and butyric acids given intraruminally at different molar proportions or individually on rumen papillae growth and IGF-I and IGFBP-3 in plasma, liver and rumen tissue in growing sheep nourished by total intragastric infusions

Song-Cheng Ma and Guang-Yong Zhao*

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, P. R. China.

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Two experiments were conducted to evaluate effects of acetic, propionic and butyric acids given intraruminally at different molar proportions or individually on the rumen papillae growth and insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3), growth hormone (GH) and insulin in plasma and/or tissues in growing sheep nourished by total intragastric infusions. The animals were daily infused with volatile fatty acids (VFA) mixtures at different molar proportions or individual VFA, casein, glucose and corn oil for a total of 1.2 times of maintenance requirement of energy and protein for 12 days. At the end of each experiment, the sheep were slaughtered and blood and tissue samples were obtained for determination of rumen papillae growth and hormones mentioned above. The present study indicated that the papillae growth and IGF-I concentration in rumen tissue of growing sheep nourished by intragastric infusions were not affected significantly by VFA mixtures with different molar proportions or by acetic, propionic and butyric acids individually ($P > 0.05$) when they were supplied at the same energy level and the synthesis and secretion of IGF-I could be more associated with the energy level rather than energy source of VFA.

Key words: Acetic acid, propionic acid, butyric acid, IGF-I, sheep.

INTRODUCTION

Rumen epithelium is very important for ruminant animals for the physiological functions of nutrient absorption and metabolism and so is its development. Although the physical stimulation of feed may account for the increase of rumen mass, the presence of volatile fatty acids (VFA) from ruminal fermentation is required for the development of rumen epithelium (Baldwin et al., 2004). Previous studies indicated that the stimulation to rumen epithelial development by VFA seemed to be mediated by several hormones (Neogrady et al., 1989; Baldwin, 1999) since such hormones like insulin, insulin-like growth factor-I (IGF-I) and epidermal growth factor showed the stimulation to the proliferation of ruminal epithelium *in vivo* and overcame the inhibition of VFA *in vitro* (Sakata et al., 1980; Baldwin, 1999). However, the hormones responsible for the rumen epithelial proliferation have not yet been delineated (Baldwin et al., 2004). IGF-I is believed to be the key regulator of growth and widely involved in the proliferation and differentiation of multiple cell types (Cohick and Clemmons, 1993). Therefore, it would be meaningful to understand the role of VFA in the rumen cell development when the regulation of IGF-I synthesis and secretion by VFA is clear. In our previous work, we

*Corresponding author. E-mail address: zhaogy@cau.edu.cn.

Abbreviations: IGF-I, Insulin-like growth factor-I; **IGFBP-3,** I insulin-like growth factor binding protein-3; **GH,** growth hormone; **VFA,** volatile fatty acids.

Table 1. Components of VFA stock solutions.

Stock solutions	Acetic acid (g)	Propionic acid (g)	Butyric acid (g)	H_2O (g)	$CaCO_3$ (g)
I	45.1	433.9	79.4	423.6	18.0
II	133.7	397.1	58.1	393.1	18.0
III	276.3	327.0	33.1	345.6	18.0
IV	363.6	284.1	17.8	316.5	18.0
V	798.6	0.0	0.0	183.4	18.0
VI	0.0	560.6	0.0	421.4	18.0
VII	0.0	0.0	468.3	513.7	18.0

All the stock solutions had the same energy concentration of 11.66 kJ per kg. For Experiment 1, VFA stock solutions I, II, III and IV were prepared from acetic, propionic and butyric acids at the molar proportion of 10: 78: 12, 27: 65: 8, 49: 47: 4 and 60: 38: 2, respectively. For Experiment 2, VFA stock solutions V, VI and VII were prepared from acetic, propionic and butyric acids individually.

studied the effect of the mixture of acetic, propionic and butyric acids (molar proportion of 65: 25: 10) on IGF-I synthesis and secretion in growing sheep and the results showed that the IGF-I concentration in plasma and tissues were increased with increasing infusion level of VFA mixture (Zhao and Sun, 2009). In normally-fed ruminants, however, the molar proportion of different VFA from ruminal fermentation varies widely depending on feed types and treatment methods. Thus, two experiments were carried out in the present study to evaluate effects of acetic, propionic and butyric acids given intraruminally at different molar proportions or individually on rumen papillae size, plasma growth hormone (GH), insulin and IGF-I and IGFBP-3 in plasma, liver and rumen tissues in growing sheep nourished by total intragastric infusions.

MATERIALS AND METHODS

Animals and treatments

The animal management in this study was approved by the Animal Care and Use Committee of China Agricultural University.

In Experiment 1, twelve four-month-old Dorset × Small-Tail-Han male sheep (24.9 ± 0.4 kg) each fitted with a rumen fistula and an abomasal catheter were randomly allocated into four groups with three animals in each group and were intraruminally infused the mixtures of acetic, propionic and butyric acids with four different molar proportions of 10: 78: 12, 27: 65: 8, 49: 47: 4 and 60: 38: 2, respectively. The total daily infusion levels of energy and protein were both 1.2 times of the maintenance (M) requirements of 450 kJ and 350 mg nitrogen (N) per kg of $BW^{0.75}$ (Ørskov et al., 1979; MacLeod et al., 1982). Casein served as the sole protein source while the energy was derived from VFA, casein, glucose and corn oil, which respectively provided 0.74, 0.16, 0.15 and 0.15 M of energy.

In Experiment 2, twelve four-month-old Dorset × Small-Tail-Han male sheep (21.7 ± 0.4 kg) each fitted with a rumen fistula and an abomasal catheter were randomly allocated into three groups with four animals in each group and were intraruminally infused acetic acid, propionic acid or butyric acid individually. The total daily infusion levels of energy and protein were the same as in Experiment 1. Casein served as the sole protein source while the energy was derived from VFA, casein, glucose and corn oil, which respectively provided 0.44, 0.16, 0.3 and 0.3 M of maintenance energy.

Solution preparation and infusion

The stock solutions of buffer, casein, mineral, vitamin mixture and trace element were prepared as described previously (Sun and Zhao, 2009) with the exception of VFA stock solutions (Table 1) and were diluted to infusion level using distilled water (Table 2). The total daily infusion weight was 0.53 kg per kg of $BW^{0.75}$, of which the VFA and buffer infusates into rumen accounted for one and two fourths, respectively, and the infusate into abomasum accounted for another one fourth. The calculated mineral stock solution was added into VFA stock solution and the infusate into abomasum was composed of casein stock solution, trace element stock solution, glucose, corn oil and distilled water. Each solution prepared was well mixed before infusion and the daily infusion was carried out between 9:00 a.m. to 9:00 p.m. in the 12-day experimental period. Multi-channel peristaltic pumps were used for infusion and the infusion procedure was performed as described previously (Sun and Zhao, 2009).

Sampling and determination

Blood samples were taken from jugular vein into vacuum tubes containing heparin in the morning of the thirteenth day before the sheep were slaughtered and plasma was obtained after centrifugation at 2100 × g for 15 min at room temperature. Approximately 60 g of left posterior lobe of liver, 30 g of rumen dorsal sac and 30 g of rumen ventral sac were sampled immediately after the sheep were slaughtered. About 1 cm^2 of fresh rumen tissue was cut for the preparation of paraffin section (Shen et al., 2004) and Motic digital medical image analysis system (Motic Ltd., China) was used to evaluate the papillae length, width and density. All the samples for other parameters were stored in a freezer at -80℃ until analysis. Radioimmunoassay was applied to determine IGF-I, IGFBP-3, GH and insulin with commercially available kits (DSL-2800, 6600, 1900 and 1600, respectively; Diagnostic Systems Laboratories Inc., USA). IGF-I and IGFBP-3 were extracted by acid-ethanol cryoprecipitation and the kits used were validated for the ovine plasma and tissues by running the standard curves prior to analysis. Plasma glucose and total protein content in tissues were determined colorimetrically with the commercially available kits from Sino-UK Institute of Biological Technology (HY-706 and 718, respectively; Beijing, China). All the data were permitted with the intra- and interassay coefficient of variation below 10%.

Table 2. Requirements of the stock solutions and other components.

Name	Calculations (g)	Notes
VFA stock solution	450 × kg $BW^{0.75}$ × *a* 11.66	*a* refers to 0.74 and 0.44 in Exp 1 and 2, respectively.
Casein stock solution	350 × kg $BW^{0.75}$ × 1.2/0.0122 × 0.001	Nitrogen content of casein stock solution is 0.0122 g N per g.
Buffer stock solution	3.7 × (450 × kg $BW^{0.75}$ × *a*)/11.66	3.7 folds of VFA stock solution.
Mineral stock solution	kg $BW^{0.75}$ × 1.4	1.4 is a coefficient.
Trace elements stock solution	kg $BW^{0.75}$ × 1.0	1.0 is a coefficient.
Glucose	450 × kg $BW^{0.75}$ × *b*/16.0	*b* refers to 0.15 and 0.30 in Experiment 1 and 2, respectively.
Corn oil	450 × kg $BW^{0.75}$ × *b*/39.0	Energy contents of glucose and corn oil are 16.0 and 39.0 k Jper g, respectively.

Table 3. Rumen papillae growth in sheep given VFA mixtures with different molar proportions (Experiment 1).

Rumen papillae size	Treatments (molar proportions of acetic, propionic and butyric acids)				P value
	10: 78: 12	27: 65: 8	49: 47: 4	60: 38: 2	
Rumen dorsal sac					
Length, mm	2.29 ± 0.17	2.75 ± 0.20	3.13 ± 0.08	2.35 ± 0.34	0.085
Width, mm	0.60 ± 0.03	0.61 ± 0.04	0.74 ± 0.08	0.82 ± 0.09	0.136
Density, n/cm^2	88 ± 5	88 ± 4	85 ± 3	93 ± 6	0.586
Rumen ventral sac					
Length, mm	2.74 ± 0.22	2.33 ± 0.31	2.67 ± 0.27	2.36 ± 0.34	0.523
Width, mm	0.66 ± 0.05	0.91 ± 0.10	0.82 ± 0.07	0.80 ± 0.10	0.262
Density, n/cm^2	95 ± 4	96 ± 3	90 ± 4	98 ± 6	0.600

Data in the same row labeled with different superscripts mean significant difference ($P < 0.05$) and tended to be different at $0.05 \leq P < 0.10$.

Statistical analysis

Analysis of variance was conducted using one-way ANOVA procedure of SPSS 10.0 (SPSS Inc., USA) to compare the effects of different treatments. The individual sheep was considered as the experimental unit. Results were presented as mean ± S.E.M. (standard error of mean) and differences between treatments were considered to be significant when P value < 0.05 and tended to be significant at $0.05 \leq P < 0.10$.

RESULTS

Data in the sheep given VFA mixtures with different molar proportions (Experiment 1) are listed in Tables 3 and 4. The results indicated that there was no significant difference in rumen papillae growth and IGF-I in plasma, liver and rumen tissues between different treatments ($P > 0.05$) while papillae length in the rumen dorsal sac tending to be different ($0.05 \leq P < 0.10$). The plasma concentrations of IGFBP-3, GH, insulin and glucose were not significantly different between different treatments ($P > 0.05$) while IGFBP-3 concentration showed diverse significant differences in rumen dorsal sac and ventral sac.

Data in the sheep given acetic, propionic and butyric acids individually (Experiment 2) are listed in Tables 5 and 6. One sheep in the treatment given butyric acid showed the symptoms of acidosis and was thus removed from the study. Therefore, three normal sheep in this treatment and four sheep in other two treatments were used for statistical analysis. The results showed that rumen papillae density, IGF-I and IGFBP-3 in plasma and different tissues were not affected significantly by different treatments ($P > 0.05$), while papillae length and width in the rumen dorsal sac and papillae width in the rumen ventral sac tended to be different significantly ($0.05 \leq P < 0.10$). Plasma GH, insulin and glucose were not significantly different between different treatments ($P > 0.05$).

DISCUSSION

Effects of different molar proportions of acetic, propionic and butyric acids on rumen papillae size and IGF-I, IGFBP-3, GH and insulin in plasma and/or tissues (Experiment 1)

The somatotropic axis is well known in playing a key role

Table 4. Plasma and tissue parameters in sheep given VFA mixtures with different molar proportions (Experiment 1).

Parameters	Treatments (molar proportions of acetic, propionic and butyric acids)				P value
	10:78:12	27:65:8	49:47:4	60:38:2	
Plasma parameters					
IGF-I, ng/ml	112.64 ± 6.11	61.97 ± 14.39	109.01 ± 36.02	101.53 ± 18.71	0.386
IGFBP-3, ng/ml	22.25 ± 3.39	20.86 ± 6.95	18.79 ± 3.93	21.16 ± 6.74	0.975
GH, ng/ml	1.55 ± 0.08	1.54 ± 0.13	1.82 ± 0.04	1.71 ± 0.27	0.556
Insulin, µIU/ml	21.39 ± 5.13	15.43 ± 0.90	15.87 ± 3.21	16.86 ± 2.63	0.591
Glucose, mmol/l	5.27 ± 0.55	4.08 ± 0.51	4.07 ± 0.40	3.87 ± 0.44	0.228
Tissue IGF-I, µg/g TP[a]					
Liver	48.87 ± 6.42	37.91 ± 14.26	55.33 ± 5.92	48.16 ± 13.70	0.728
Rumen dorsal sac	46.14 ± 5.63	37.08 ± 3.75	56.54 ± 8.68	56.15 ± 8.21	0.221
Rumen ventral sac	36.19 ± 4.53	30.65 ± 4.40	63.47 ± 14.05	61.02 ± 12.67	0.105
Tissue IGFBP-3, µg/g TP					
Liver	6.46 ± 1.54	16.31 ± 4.69	17.19 ± 5.64	8.51 ± 1.48	0.190
Rumen dorsal sac	11.88 ± 2.99	6.57 ± 1.21	11.55 ± 3.82	21.71 ± 4.79	0.076
Rumen ventral sac	10.57 ± 1.07[a]	5.87 ± 0.27[a]	19.47 ± 3.52[b]	9.58 ± 0.66[a]	0.005

[a] µg/g TP, µg per gram of total protein in tissue. The four treatments were given VFA mixtures with the molar proportion of 10:78:12, 27:65:8, 49:47:4 and 60:38:2, respectively. Data in the same row labeled with different superscripts mean significant difference ($P < 0.05$) and tended to be different at $0.05 \leq P < 0.10$.

Table 5. Rumen papillae growth in sheep given acetic acid, propionic acid and butyric acid individually (Experiment 2).

Rumen papillae size	Treatments (VFA)			P value
	Acetic acid	Propionic acid	Butyric acid	
Rumen dorsal sac				
Length, mm	2.27 ± 0.29	1.99 ± 0.14	2.94 ± 0.22	0.060
Width, mm	0.64 ± 0.02	0.51 ± 0.06	0.50 ± 0.04	0.099
Density, n/cm^2	86 ± 5	86 ± 5	92 ± 4	0.662
Rumen ventral sac				
Length, mm	2.40 ± 0.29	2.24 ± 0.18	2.86 ± 0.21	0.253
Width, mm	0.78 ± 0.05	0.59 ± 0.05	0.66 ± 0.06	0.089
Density, n/cm^2	92 ± 2	95 ± 6	94 ± 4	0.919

Data in the same row labeled with different superscripts mean significant difference ($P < 0.05$) and tended to be different at $0.05 \leq P < 0.10$.

in the regulation of animal growth and development.

GH is involved in the regulation of circulating IGF-I and IGFBP-3. Meanwhile, the effects of GH in peripheral tissues are in large part mediated by IGF-I. Although active in many tissues through endocrine, autocrine and/or paracrine ways, IGF-I is synthesized primarily in liver and preserved by its carrier protein IGFBP-3 in the circulation (Clemmons and Underwood, 1991). The present results suggested that there was no marked effect of VFA mixtures with different molar proportions on liver and plasma IGF-I and plasma GH was also not affected by different VFA mixtures. It is generally recognized that both the synthesis of IGF-I in liver (Clemmons and Underwood, 1991) and the circulating GH (Breier, 1999) are primarily regulated by nutrient intake and the resulting nutritional status. There-fore, the unaffected liver and plasma IGF-I, as plasma GH were likely due to the isoenergetic VFA mixtures while the same levels of protein and energy were also offered by other nutrients. The dependence of circulating IGF-I on nutritional status was also reported in other studies in which lambs on low level of nutrient intake were accompanied by the lower plasma IGF-I (Bass et al., 1991; Hua et al., 1995). Although some variation was observed in rumen ventral sac, the IGF-I concentration in rumen tissues was not significantly affected by different molar proportions of VFA mixtures. Considering the probable role of IGF-I in cell proliferation and differentiation (Baldwin, 1999; MacDonald, 1999), it was postulated that energy level was more important than

Table 6. Plasma and tissue parameters in sheep given acetic acid, propionic acid and butyric acid individually (Experiment 2).

Parameters	Treatments (VFA)			P value
	Acetic acid	Propionic acid	Butyric acid	
Plasma parameters				
IGF-I, ng/ml	65.84 ± 2.82	88.80 ± 12.03	84.75 ± 9.69	0.203
IGFBP-3, ng/ml	39.28 ± 3.90	45.47 ± 4.52	49.90 ± 6.08	0.341
GH, ng/ml	1.98 ± 0.53	1.99 ± 0.36	1.12 ± 0.19	0.330
Insulin, μIU/ml	12.74 ± 1.60	10.55 ± 0.96	11.63 ± 0.71	0.460
Glucose, mmol/l	4.88 ± 0.87	5.30 ± 0.15	3.59 ± 0.13	0.169
Tissue IGF-I, μg/g TP[a]				
Liver	10.38 ± 0.94	9.47 ± 1.97	8.43 ± 0.27	0.653
Rumen dorsal sac	10.42 ± 1.28	8.92 ± 0.83	8.23 ± 2.99	0.656
Rumen ventral sac	8.45 ± 1.69	9.80 ± 1.38	13.52 ± 4.67	0.418
Tissue IGFBP-3, μg/g TP				
Liver	3.40 ± 0.56	2.54 ± 0.57	4.63 ± 0.77	0.127
Rumen dorsal sac	2.86 ± 0.61	4.73 ± 0.52	4.31 ± 0.70	0.115
Rumen ventral sac	7.29 ± 1.15	4.03 ± 0.65	4.46 ± 1.32	0.100

[a] μg/g TP, μg per gram of total protein in tissue. The three treatments were given the infusion of acetic, propionic and butyric acids individually. Data in the same row labeled with different superscripts mean significant difference ($P < 0.05$) and tended to be different at $0.05 \leq P < 0.10$.

molar proportion of VFA mixtures for rumen development. Although some data tended to be different ($0.05 \leq P < 0.10$), the present results indicated that the rumen papillae length, width and density were not affected significantly by different VFA mixtures when they were supplied at the same energy level. This would be particularly significant for the ruminants with varying dietary quality and quantity. For its high affinity and abundance, IGFBP-3 serves as the carrier and preserver of IGF-I in the circulation and is widely involved in the modulation of IGF-I bioactivity at the cellular level (Cohick and Clemmons, 1993). The results showed that similar concentration of plasma IGFBP-3 appeared in concert with the unaffected plasma IGF-I and the variance in tissue IGFBP-3 could be associated with their different participation in modulation of IGF-I actions at specific tissues (Cohick and Clemmons, 1993). The results suggested that plasma insulin and glucose were not affected significantly by VFA mixtures with different molar proportions. This could likely be due to the same energy intake and the regulation of glucose metabolism by insulin (Harmon, 1992).

For ruminants, insulin serves as the primary hormonal regulator of glucose metabolism to keep the glucose homeostasis according to the nutritional status (Brockman and Laarveld, 1986). Gluconeogenesis means the major source of glucose for normally-fed ruminants and propionate was thought to be the principal precursor for gluconeogenesis (Bergman, 1990). However, the contribution of propionate to blood glucose via gluconeogenesis could probably be covered by the glucose infused into the abomasum.

Effects of acetic, propionic and butyric acids given individually on rumen papillae size, and IGF-I, IGFBP-3, GH and insulin in plasma and/or tissues (Experiment 2)

The present results showed that liver and plasma IGF-I was not affected significantly by different acid infusions. For the dependence of somatotropic axis on the nutrient intake and the resulting nutritional status (Thissen et al., 1994), it seemed that the same energy level rather than the individual acid itself may be responsible for the similar IGF-I synthesis and secretion. Meanwhile, papillae length, width, density and IGF-I concentration in rumen tissues were also not affected significantly by different VFA infusions. This was in agreement with the previous reports by Shen et al. (2004) that rumen proliferation caused by an energy-rich diet was accompanied by increased plasma IGF-I level in young ruminants. Since the underlying hormones responsible for the mediation of rumen epithelial proliferation were still not clear and IGF-I was considered as one of the probable hormones (Baldwin, 1999), it seemed that rumen cell development may not be stimulated differently by the three individual acids supplied at the same energy level. It was interesting to find that coordination also appeared in this experiment between plasma IGFBP-3 and IGF-I. The variance of IGFBP-3 in different tissues also supported its contribution to the modulation of IGF-I in the specific tissues (Cohick and Clemmons, 1993).

No marked difference was found in the mean concentration of plasma glucose. Although propionate was thought to be the major glucogenic precursor (Bergman,

1990), constant gluconeogenesis in liver was essential to keep glucose homeostasis and more glucose infusion could be another contribution. Besides, the participation in regulation of postnatal growth, GH also exerts impact on glucose metabolism indirectly by altering the sensitivity of tissues to insulin (Brockman and Laarveld, 1986). In concert with the nutrient intake level, the plasma levels of GH and insulin were unaffected significantly by the three individual acids. In the previous studies, glucose seemed more than VFA to be involved in the regulation of insulin secretion (Tao and Asplund, 1975) while another research suggested that the changing ruminal VFA concentration could probably provide the physiological signals to the regulation of insulin secretion (Matsunaga et al., 1999). Since insulin was physiologically integrated with energy and protein metabolism (Tao and Asplund, 1975), taken together with our results, insulin secretion seemed to be more regulated integrally by the nutrient energy level.

Conclusion

In conclusion, the present study indicated that the papillae size and IGF-I concentration in rumen tissues of growing sheep nourished by intragastric infusions were not affected significantly by VFA mixtures with different molar proportions or by acetic, propionic and butyric acids individually when they were supplied at the same energy level and the synthesis and secretion of IGF-I could be more associated with the energy level rather than energy source of VFA.

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