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Full Length Research Paper

Effects of mixed volatile fatty acid sodium salt on insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein-3 (IGFBP-3) in plasma and rumen tissue, and rumen epithelium development in lambs

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Fifteen cross-bred male lambs (Dorset × Small Tail *Han*), aged 7 weeks, with average liveweight of 13.8±0.8 kg, were used to study effects of feeding mixed volatile fatty acid (VFA) sodium salt on insulin-like growth factor-I (IGF-I), insulin-like growth factor-binding protein-3 (IGFBP-3) in plasma and rumen tissue and rumen epithelium development. The lambs were randomly divided into 5 groups with 3 lambs in each group. Graded levels of mixed VFA sodium salt (the molar proportion of acetate, propionate, and butyrate was 65:25:10), i.e. 0, 5, 15, 30 and 60g/d was added into milk replacer as experimental treatments I, II, III, IV and V, respectively. The trial lasted 24 days. No differences were found in plasma IGF-I and IGFBP-3 between different treatments (P>0.05), or in IGF-I and IGFBP-3 in rumen dorsal sac and in IGF-I in ventral sac between different treatments (P>0.05), while IGFBP- 3 of treatments IV and V was higher than other treatments in ventral sac (*P*<0.05). In rumen dorsal sac, the length and density of rumen papillae of treatments III, IV and V were higher than treatment I (*P*<0.05) while no differences were found in the width and surface area of rumen papillae between different treatments or in length, width, density and surface area of rumen papillae in ventral sac between different treatments (P>0.05). It was concluded that feeding mixed VFA sodium salt up to 60 g/d per lamb in milk replacer had no significant effect on development of rumen epithelium.

Key words: volatile fatty acid sodium salt; insulin-like growth factor-I (IGF-I); insulin-like growth factor-binding protein-3 (IGFBP-3); rumen epithelium; lambs.

INTRODUCTION

Many studies indicated that volatile fatty acids (VFA) and VFA salts stimulated rumen epithelium growth. In lambs aged 2 to 12 weeks, ruminal infusion of mixed VFA at a level of 12.5 to 50% of the energy required tends to increase the length of rumen papillae and stimulate the metabolic development of the rumen (Lane and Jesse, 1997). In adult sheep, ruminal dosing of sodium propionate or sodium acetate solution with 18 mmol/kg body weight daily increased the mitotic index of the rumen epithelial cells (Sakata and Tamate, 1979). In growing sheep nourished by intragastric infusions, ruminal infusion of VFA mixtures with different molar proportions or acetic, propionic and butyric acids individually at the same level of energy supply did not affect the papillae growth and

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

insulin-like growth factor-I (IGF-I) concentration in rumen tissue (Ma and Zhao, 2010), whereas IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), growth hormone (GH) and insulin in plasma and IGF-I, IGFBP-3 in rumen epithelium increased with the level of ruminal infusion of mixed VFA (molar proportion of acetic acid, propionic acid and butyric acid was 65:25:10) (Zhao and Sun, 2010). Since ruminal infusion of insulin (0.125 U/kg/h) plus glucose (300 mg/kg/h) stimulated mitotic index of rumen epithelium in adult sheep (Sakata et al., 1980), and insulin and IGF-I were effective inducers of rumen epithelium growth *in vitro* (Baldwin, 1999), the stimulation of rumen epithelium development of sheep by VFA could be more associated with the VFA energy level (Ma and Zhao, 2010) and mediated by IGF-I, insulin and other hormones. The objective of this experiment is to study the effects of feeding graded levels of mixed VFA sodium salt on IGF-I, IGFBP-3, insulin, GH in plasma and IGF-I and IGFBP-3 in rumen tissue and development of rumen epithelium, and further study the relationships between VFA sodium salt level and IGF-I and IGFBP-3 in lambs.

MATERIALS AND METHODS

Animals, experimental design and feeding

Fifteen cross-bred male lambs (Dorset × Small Tail *Han*) , aged 7 weeks, with average liveweight of 13.8 ± 0.8 kg were used as experimental animals. The lambs (13.8 ± 0.8 kg) were randomly divided into 5 groups with 3 lambs in each group. Milk replacer powder (Beijing Precision Animal Nutrition Centre, Beijing, China) was fed to the lambs as the basal nutrient supply. Graded levels of mixed VFA sodium salt (the molar ratio of acetate, propionate and butyrate was 65:25:10), that is, 0, 5, 15, 30 and 60 g/day, were added to milk replacer of each group, as experimental treatments I, II, III, IV and V, respectively. The lambs were housed in individual metabolic cages and were fed 300 g milk replacer/day/lamb. The milk replacer was divided into two equal meals and fed at 8:00 and 17:00, respectively. The milk replacer and the mixed VFA sodium salt were dissolved and well mixed in warm water (33 to 35°C) and then the suspension was fed to the lambs with buckets. The feeding trial lasted for 24 days and the lambs had free access to drinking water during the trial.

The milk replacer was composed of milk powder, whey powder, mixed amino acids, calcium hydrogen phosphate ($CaHPO_4$), sodium chloride (NaCl), calcium carbonate ($CaCO_3$), vitamin A, vitamin D3, vitamin E and ethoxy quinoline, The dry matter (DM) content of the milk replacer was 920 g/kg. It contained 120 g ether extract, 230 g crude protein, 8 g total phosphorus, 25 g lysine, 15 g methionine, 12 g threonine, 4 g sodium chloride, 15000 IU vitamin A, 2500 IU vitamin D3, 80 IU vitamin E and 10 mg ethoxy quinoline per kg DM.

Sampling and analysis

On the last day of the feeding trial, blood samples were taken from jugular vein into vacutainers containing heparin. Plasma was obtained after centrifugation at 2100 ×g for 15 min at room temperature and stored at -20°C until analysis. Then the lambs were slaughtered and the rumen was evacuated and rinsed with warm tap water to remove digesta. About 1 cm^2 of rumen dorsal sac and ventral sac was sampled and the samples were fixed in 4% formaldehyde solution and kept at -80°C.

The length and width of rumen epithelium papillae were determined using computer-operated MOTIC microscope dmb B5 series picture analysis system (INTRONIC GmbH, Berlin, Germany) and minimal invasive aortic surgery system (University of Aeronautics and Astronautics, Beijing, China). The papillae density (number of papillae/cm^2 mucosa) was estimated using a digital camera (OLYMPUS E30) and a light microscope (BM OPTICAL, Shanghai, China). The total surface area of papillae/cm^2 mucosa was calculated as length x width x 2, multiplied by the number of papillae/cm^2.

IGF-I, IGFBP-3, GH, insulin and glucagon were analyzed using radioimmunoassay with commercially available kits (HY-60, Sino-UK Institute of Biological Technology, Beijing, China; DSL-2800, 6600, 1900 and 1600, respectively; Diagnostic Systems Laboratories Inc., USA). Plasma glucose and total protein content in tissues were analysed using the commercially available kits (HY-706 and 718, Sino-UK Institute of Biological Technology, Beijing, China).

Statistical analysis

Dose effects were analysed using the one-way analysis of variance (ANOVA) (SPSS 13.0). Differences were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

No difference was found in plasma IGF-I and IGFBP-3 ($P > 0.05$) (Table 1), or in IGF-I and IGFBP-3 in rumen dorsal sac and IGF-I in rumen ventral sac between different treatments ($P > 0.05$), or in insulin, glucagon, GH and glucose in plasma between different treatments, whereas, IGFBP-3 in treatments IV and V was higher than other treatments ($P < 0.05$) (Table 2).

In rumen dorsal sac, the length of rumen papillae was decreased ($P < 0.01$) and the densities of rumen papillae was increased ($P < 0.05$) with the mixed VFA sodium salt level, while no difference was found in the width and surface area of rumen papillae between different treatments. No difference was found in the length, width, density and surface area of rumen papillae between different treatments ($P > 0.05$) (Table 3).

Effects of mixed VFA sodium salt on plasma IGF-I, IGFBP-3, GH, insulin, glucagon, glucose, and rumen IGF-I and IGFBP-3

IGF-I is synthesized in liver and preserved by its protein carrier IGFBP-3 in circulation (Clemmons and Underwood, 1991). Many researchers reported that IGF-I and IGFBP-3 levels were related to nutritional status of animals. Lower plasma IGF-I in lambs was accompanied with low nutrient intake (Bass et al., 1991), plasma IGF-I in young goats was higher with an energy-rich diet (Shen et al., 2004) and plasma IGF-I, IGFBP-3 in growing sheep increased significantly with ruminal infusion of VFA level (Zhao and

Table 1. Effects of mixed VFA sodium salt on plasma and rumen IGF-I and IGFBP-3 in lambs.

Parameter	Mixed VFA salt added (g/day/lamb)					P value
	0	5	15	30	60	
Energy supply						
Milk replacer (KJ/day)	5787	5787	5787	5787	5787	-
VFA salt (KJ/day)	0	65.3	195.9	391.8	783.6	-
Total energy supply (KJ/day)	5787	5852.3	5982.9	6178.8	6570.6	-
VFA salt energy/total energy supply (%)	0	1.12	3.27	6.34	11.93	-
Plasma						
IGF-I (ng/ml)	247.78±27.74	228.64±24.44	240.91±26.98	496.30±116.64	329.12±69.86	0.069
IGFBP-3 (ng/ml)	33.13±2.42	36.83±6.76	33.10±3.68	40.59±8.69	25.31±7.04	0.529
Rumen dorsal sac						
IGF-I (μg/g TP*)	96.59±11.37	102.73±2.93	94.10±3.17	130.48±34.84	150.98±14.56	0.178
IGFBP-3 (μg/gTP)	20.50±1.77	27.52±13.20	20.40±1.28	23.24±4.54	33.90±583	0.612
Rumen ventral sac						
IGF-I (μg/gTP)	104.45±6.06	123.43±22.48	112.83±10.74	155.61±8.10	144.21±5.95	0.071
IGFBP-3 (μg/gTP)	25.74±4.23^{a}	23.80±5.48^{a}	28.10±5.02^{a}	34.23±4.21^{b}	46.98±2.72^{b}	0.025

*μg/g TP, μg per gram of total protein in tissue. Values are means± SEM. Data in the same row labeled with different lowercase superscripts mean significant difference (P<0.05).

Sun, 2010). In the present experiment, it was found that feeding mixed VFA sodium salt did not affect IGF-I and IGFBP-3 in plasma, in rumen dorsal sac and IGF-I in rumen ventral sac, but IGFBP-3 of treatments IV and V in rumen ventral sac was significantly higher than treatments I, II and III (P<0.05) when VFA salt levels were 30 and 60 g/d per lamb. GH circulation was regulated by nutrient intakes in farming animals (Breier, 1999). Plasma GH in growing sheep was increased significantly with ruminal infusion of VFA level (Zhao and Sun, 2010), whereas plasma GH was not affected by different molar proportions of VFA when the energy supply was kept at the same level (Ma and Zhao, 2010). In the present experiment, feeding different levels of mixed VFA sodium salt in milk replacer did not significantly affect plasma GH. The result was not in agreement with the results of Zhao and Sun (2010). Insulin and glucagon in plasma increased significantly with the VFA infusion level (Zhao and Sun, 2010), while infusion of isoenergetic VFA mixtures had no effects on insulin and glucose (Ma and Zhao, 2010), indicating that energy level was crucial for the influence on these parameters. In the present experiment, feeding mixed VFA sodium salt up to 60 g/d per lamb did not affect plasma insulin, glucagon and glucose. The reason for the differences between results of the present experiment and other studies could be that the energy percentages of mixed VFA sodium salt in the present experiment were 0, 1.12, 3.27, 6.34 and 11.92% of the total energy supply for groups I, II, III and IV, respectively, which might not be high enough to influence these parameters.

Effects of mixed VFA sodium salt on development of rumen epithelium

Development of rumen epithelium was stimulated by VFA infusion (Lane and Jesse, 1997) and dietary change (Shen et al., 2004). Ruminal infusion of VFA to growing sheep significantly increased IGF-I and IGFBP-3 in plasma and rumen

Table 2. Effects of mixed VFA sodium salt on plasma hormones and glucose in lambs

Parameter	Mixed VFA salt added, (g/d/lamb)					P value
	0	5	15	30	60	
Insulin (μIU/ml)	16.32±1.04	20.45±0.87	18.00±0.39	15.58±1.64	18.82±1.26	0.070
Glucagon (ng/ml)	141.42±9.02	125.71±4.05	130.43±10.33	135.75±11.83	145.35±6.10	0.536
GH (ng/ml)	5.36±0.76	4.35±0.42	4.28±0.25	5.64±1.25	5.00±0.51	0.641
Glucose (mmol/L)	3.56±0.18	3.42±0.13	3.30±0.07	3.87±0.16	3.59±0.24	0.237

Values are means± SEM. Data in the same row labeled with different lowercase superscripts mean significant difference (P<0.05).

Table 3. Effects of mixed VFA sodium salt on rumen papillae in lambs

Parameter	Mixed VFA salt added, (g/d/lamb)					*P* value
	0	5	15	30	60	
Rumen dorsal sac						
Length (mm)	2.45±0.32^{a}	1.83±0.13^{ab}	1.21±0.06^{b}	1.73±0.30^{b}	1.21±0.09^{b}	0.010
Width (mm)	0.51±0.05	0.60±0.04	0.57±0.06	0.52±0.04	0.69±0.13	0.422
Density (n/cm^2)	100±3^{a}	107±4^{ab}	112±3^{bc}	118±2^{bc}	120±5^{bc}	0.019
Surface area (mm^2/cm^2)	262.27±66.64	234.10±5.68	155.20±10.85	208.26±31.44	196.55±29.86	0.350
Rumen ventral sac						
Length (mm)	2.03±0.13	2.09±0.38	1.63±0.27	2.01±0.14	1.93±0.11	0.654
Width (mm)	0.68±0.08	0.59±0.04	0.65±0.05	0.61±0.03	0.67±0.06	0.724
Density (n/cm^2)	93±5	94±4	102±2	107±4	101±4	0.132
Surface area (mm^2/cm^2)	257.33±33.40	235.98±55.62	212.78±31.26	259.08±11.10	263.77±42.38	0.858

Values are means± SEM. Data in the same row labeled with different lowercase superscripts mean significant difference (P<0.05).

rumen tissue (Zhao and Sun, 2010). Since IGF-I is an important hormone that promotes the tissue proliferation and differentiation, the stimulation of development of rumen epithelium by VFA may be mediated by IGF-I and IGFBP-3. In the present experiment, it was found that that addition of mixed VFA sodium salt had no significant effects on development of rumen epithelium. The reason for this could be that the intake of milk replacer of all treatments was the same and the energy of mixed VFA sodium salt added was relatively low compared to the total energy supply. The results were in agreement with the unaffected IGF-I and IGFBP-3 in plasma and rumen dorsal sac.

The relationships between mixed VFA sodium salt and IGF-I, IGFBP-3 in rumen epithelium

Significant correlations were found between mixed VFA sodium salt (x, g/d) and IGF-I content (y, μg/g TP) in rumen dorsal sac: $y = 0.97x + 93.72$, $r^2 = 0.388$, $n = 15$, $P < 0.05$, between mixed VFA sodium salt (x, g/d) and IGF-I content (y, μg/g TP) in rumen ventral sac: $y = 0.66x + 113.58$, $r^2 = 0.304$, $n = 15$, $P < 0.05$, and between mixed VFA sodium salt (x, g/d) and IGFBP-3 content (y, μg/g TP) in rumen ventral sac: $y = 0.38x + 23.35$, $r^2 = 0.623$, $n = 15$, $P < 0.01$. No significant relationship was found between mixed VFA sodium salt and other parameters. On one hand, the relationship indicated that mixed VFA sodium salt influenced IGF-I in rumen ventral sac and dorsal sac and IGFBP-3 in ventral sac; on the other hand, the low regression coefficients (r^2) indicated high variation of IGF-I and IGFBP-3 in rumen tissue among different lambs.

Conclusion

Feeding 30-60 g mixed VFA sodium salt/d per lamb in milk replacer increased IGFBP-3 in rumen ventral sac, but did not significantly affect IGF-I and IGFBP-3 in plasma and in rumen dorsal sac, and IGF-I in rumen ventral sac. The length of rumen papillae decreased and the density of rumen papillae increased with mixed VFA sodium salt level whereas no influence was found on other parameters

of rumen papillae. Feeding mixed VFA sodium salt up to 60 g/d per lamb had no significant effect on development of rumen epithelium. It may be necessary to study the effect of higher levels of VFA salts on IGF-I and IGFBP-3 in plasma and rumen tissue and on development of rumen epithelium in lambs in the future.

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