



## Effects of Volatile Fatty Acids on IGF-I, IGFBP-3, GH, Insulin and Glucagon in Plasma, and IGF-I and IGFBP-3 in Different Tissues of Growing Sheep Nourished by Total Intragastric Infusions\*

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**ABSTRACT :** Twelve Suffolk×Small-tail-*Han* male sheep (body weight 21-26 kg), aged four months, were used to study the effects of volatile fatty acids (VFA) on IGF-I (insulin-like growth factor-I), IGFBP-3 (insulin-like growth factor binding protein-3), GH (growth hormone), insulin and glucagon in plasma, and IGF-I and IGFBP-3 in different tissues. The sheep were randomly divided into four groups with 3 sheep in each group. The sheep were sustained by total intragastric infusions and four levels of mixed VFA (the molar proportion of acetic acid, propionic acid and butyric acid was 65:25:10), which supplied 333, 378, 423 and 468 KJ energy/kg W<sup>0.75</sup>/d, were infused into the rumen as experimental Treatments I, II, III and IV, respectively. The experiment lasted 12 days, of which the first 8 days were for pretreatment and the last 4 days for collection of samples. At the end of the experiment, blood samples were taken and then the sheep were slaughtered and tissue samples from the rumen ventral sac, rumen dorsal sac, liver, duodenum and *Longissimus dorsi* muscle were obtained. IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and IGF-I and IGFBP-3 in different tissues were analysed. Results showed that the concentration of IGF-I, IGFBP-3, GH, insulin or glucagon in plasma and the content of IGF-I and IGFBP-3 in the rumen dorsal sac, rumen ventral sac, liver or *Longissimus dorsi* muscle were increased with VFA infusion level ( $p < 0.05$ ). No significant differences were found in duodenum IGF-I between Treatments I and II and in rumen dorsal sac IGFBP-3 between Treatments II and III ( $p > 0.05$ ). It was concluded that IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and IGF-I and IGFBP-3 in rumen dorsal sac, rumen ventral sac, liver and *Longissimus dorsi* muscle were increased significantly with increasing level of ruminal infusion of mixed VFA. (**Key Words :** Volatile Fatty Acids, IGF-I, IGFBP-3, Growth Hormone, Insulin, Glucagon, Sheep)

### INTRODUCTION

Volatile fatty acids (VFA) promote rumen development in calves (Sakata and Tamate, 1979) and rumen epithelial papilla growth in lambs (Lane et al., 1997). However, it was found that butyric acid and other VFA inhibited proliferation of rumen epithelium incubated *in vitro* (Kruth et al., 1982; Neogrady et al., 1989a; Baldwin, 1999). The inhibition effect of VFA, however, could be overcome by insulin-like growth factors -I (IGF-I), insulin and epidermal growth factor (EGF) (Baldwin, 1999). The difference of the VFA effect on rumen epithelium between *in vivo* and *in vitro* studies could be that VFA stimulated the secretion of IGF-I, GH and other hormones *in vivo* which in turn

improved rumen epithelium growth, whereas *in vitro*, no hormones were secreted. In order to clarify this hypothesis, study of the quantitative relationship between VFA supply and IGF-I, IGFBP-3 and GH in ruminants is needed.

Because of the diurnal variation of rumen VFA concentration and the difficulty of accurate determination of VFA production in normally-fed ruminants, the total intragastric infusion technique developed by Ørskov et al. (1979a), which allows accurate control of amount and molar proportions of VFA in ruminants, was used in the study.

The objective of the experiment was to study the effect of VFA on IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and IGF-I and IGFBP-3 in different tissues, and the relationship between VFA and IGF-I and IGFBP-3 of liver, rumen ventral sac, rumen dorsal sac, duodenum and *Longissimus dorsi* muscle of growing sheep nourished by total intragastric infusion.

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## MATERIALS AND METHODS

### Animals

Twelve Suffolk×Small-tail-*Han* male sheep (live weight 21-26 kg), aged four months and fitted with fistulae in the rumen and abomasum, were used in the experiment.

### Experimental design

The experimental design was the same as that of Sun and Zhao (2009). The sheep were divided into four groups with three sheep in each group. Four levels of mixed VFA (the molar proportion of acetic acid, propionic acid and butyric acid was 65:25:10), which supplied 333, 378, 423 and 468 KJ energy/kg  $W^{0.75}/d$ , were infused into the rumen of the sheep as Treatments I, II, III and IV, respectively. The experiment lasted for 12 days, of which the first 8 days were for pretreatment and the last 4 days for collection of samples.

### Preparation of infusates and procedures of infusion

The preparation of infusates such as VFA, casein and buffer and infusion levels were as described by Sun and Zhao (2009). The VFA, buffer and mineral solutions were infused into the rumen and the casein mixture (including glucose, corn oil, vitamins and trace elements) was infused into the abomasum. The infusion began at 9:00 a.m. and ended at 9:00 p.m. every day during the experimental period. The sheep had free access to drinking water during the experiment.

### Sampling

At the end of the experiment, a 20 ml blood sample was taken from each sheep. The blood samples were centrifuged at 3,400 g for 15 min and the plasma was obtained. Then the sheep were slaughtered and tissue samples (about 50 g) including rumen dorsal sac, rumen ventral sac, liver, *Longissimus dorsi* muscle and duodenum were obtained from each sheep. All samples were kept in a freezer at -70°C for later analysis.

### Determinations and analysis

About 1.0 g of tissue sample (rumen dorsal sac, rumen ventral sac, liver, *Longissimus dorsi* muscle and duodenum) was triturated in saline for 3 min. Then 0.4 ml (1 N) formic acid-ethanol (95% ethanol: 2 M HCl = 87.2:12.5) was added, the sample was vigorously stirred and then centrifuged at 1,500 g for 30 min. One ml of the supernatant was obtained for the determination of total protein (TP) and IGF-I. Another 1.0 g tissue sample was triturated in saline for 3 min. Then 0.5 ml (1 N) acetic acid and 0.5 ml NaOH (1 N) solution were added, stirred and centrifuged at 1,500 g for 30 min. One ml of the supernatant was obtained for

the determination of IGFBP-3.

The IGF-I, IGFBP-3, GH, insulin and glucagon concentrations in plasma and IGF-I and IGFBP-3 in tissue samples were analysed using specific radioimmunoassay kits (Diagnostic System Laboratories, USA). Before assay, IGF-I was extracted with acid-ethanol cryoprecipitation (Breier et al., 1991). The TP of the tissue samples was determined using the biuret method (reaction temperature: 37°C, wave length: 546 nm). The determinations and analysis of the samples were assisted by Sino-UK Institute of Biological Technology (Sino-UK Institute of Biological Technology, Beijing, China).

### Data analysis

The IGF-I and IGFBP-3 in different tissues were expressed on the basis of the TP content ( $\mu\text{g/g}$  TP). One way ANOVA was used for the analysis of variance among different treatments and linear correlations between different parameters were calculated using SPSS 12.0.

## RESULTS

### Sustenance of the sheep nourished by total intragastric infusions

The sheep stopped eating feed and drinking water after the infusion started and remained calm and normal during the infusion period. The rumen pH was measured every 2 h and for all sheep it was within the range of 6.1-6.8.

### Effect of VFA on IGF-I, IGFBP-3, GH, insulin, glucagon in plasma and IGF-I and IGFBP-3 in different tissues

Results are shown in Table 1. The IGF-I, IGFBP-3, GH, insulin and glucagon in plasma increased significantly with the VFA infusion level ( $p < 0.01$ ). No significant difference in plasma GH was found between Treatments II and III ( $p > 0.05$ ). The IGF-I and IGFBP-3 in the rumen dorsal sac, ventral sac, duodenum, liver and *Longissimus dorsi* muscle were also significantly increased by the VFA infusion level ( $p < 0.01$ ). However, no significant differences were found in the IGF-I in the duodenum between Treatments I and II ( $p > 0.05$ ) and in the IGFBP-3 in the rumen dorsal sac between Treatments II and III ( $p > 0.05$ ).

### Relationships between VFA infusion level and plasma hormones and between plasma hormones

Significant correlations were found between the VFA infusion level and the IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and IGF-I in liver (Table 2), and between the IGF-I in liver (ng/ml) and the IGF-I in the rumen dorsal sac, rumen ventral sac, duodenum and *Longissimus dorsi* muscle, and between the IGFBP-3 in liver and the IGFBP-3 in different tissues (Table 3).

**Table 1.** IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and IGF-I and IGFBP-3 in tissues

Item	Treatments				SE
	I	II	III	IV	
Metabolic body weight (kg)	10.89	10.33	10.33	10.67	0.27
Total VFA supply (g/d)	205.40 <sup>a</sup>	220.99 <sup>a</sup>	247.32 <sup>b</sup>	282.60 <sup>c</sup>	6.29
Plasma					
IGF-I (ng/ml)	177.99 <sup>a</sup>	217.69 <sup>b</sup>	247.15 <sup>c</sup>	297.82 <sup>d</sup>	6.11
IGFBP-3 (ng/ml)	10.86 <sup>a</sup>	24.14 <sup>b</sup>	39.50 <sup>c</sup>	48.45 <sup>d</sup>	1.22
GH (ng/ml)	2.07 <sup>a</sup>	2.76 <sup>b</sup>	3.02 <sup>b</sup>	3.81 <sup>c</sup>	0.09
Insulin (μIU/ml)	18.30 <sup>a</sup>	24.65 <sup>b</sup>	29.37 <sup>c</sup>	40.11 <sup>d</sup>	0.80
Glucagon (ng/L)	121.40 <sup>a</sup>	136.71 <sup>b</sup>	208.94 <sup>c</sup>	429.27 <sup>d</sup>	2.69
IGF-I in tissues (μg/g TP)					
Rumen dorsal sac	95.66 <sup>a</sup>	166.12 <sup>b</sup>	352.21 <sup>c</sup>	656.80 <sup>d</sup>	12.33
Rumen ventral sac	60.16 <sup>a</sup>	82.88 <sup>b</sup>	100.40 <sup>c</sup>	130.08 <sup>d</sup>	3.50
Liver	111.10 <sup>a</sup>	207.42 <sup>b</sup>	370.75 <sup>c</sup>	528.55 <sup>d</sup>	5.33
Duodenum	160.43 <sup>a</sup>	212.67 <sup>a</sup>	346.84 <sup>b</sup>	426.28 <sup>c</sup>	21.02
<i>Longissimus dorsi</i> muscle	470.86 <sup>a</sup>	599.45 <sup>b</sup>	759.54 <sup>c</sup>	957.67 <sup>d</sup>	10.63
IGFBP-3 in tissues (μg/g TP)					
Rumen dorsal sac	37.57 <sup>a</sup>	98.48 <sup>b</sup>	101.12 <sup>b</sup>	122.82 <sup>c</sup>	1.59
Rumen ventral sac	16.40 <sup>a</sup>	24.17 <sup>b</sup>	33.59 <sup>c</sup>	51.71 <sup>d</sup>	1.09
Liver	19.06 <sup>a</sup>	38.80 <sup>b</sup>	48.36 <sup>c</sup>	71.15 <sup>d</sup>	0.62
Duodenum	16.23 <sup>a</sup>	24.95 <sup>b</sup>	55.37 <sup>c</sup>	75.70 <sup>d</sup>	0.89
<i>Longissimus dorsi</i> muscle	229.16 <sup>a</sup>	304.53 <sup>b</sup>	368.59 <sup>c</sup>	455.18 <sup>d</sup>	8.21

Data labeled with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Treatments I, II, III and IV refer to 333, 378, 423 and 468 kJ energy/kg  $W^{0.75}$ /d from VFA, respectively.

VFA refers to volatile fatty acids; IGF-I = Insulin-like growth factor-I; IGFBP-3 = Insulin-like growth factor binding protein-3; GH = Growth hormone; TP = Total protein.

**Table 2.** Relationships between VFA infusion (g/d) and IGF-I, IGFBP-3, GH, insulin and glucagon in plasma (ng/ml) and IGF-I in liver (μg/g TP)

VFA infused (x)	Hormones (y)	Equations	n =	r <sup>2</sup> =	p <
VFA	IGF-I	$y = 1.31x - 78.71$	12	0.81	0.01
VFA	IGFBP-3	$y = 0.44x - 73.48$	12	0.85	0.01
VFA	GH	$y = 0.02x - 1.64$	12	0.85	0.01
VFA	insulin	$y = 0.24x - 30.24$	12	0.86	0.01
VFA	glucagon	$y = 3.68x - 655.99$	12	0.84	0.01
VFA	IGF-I in liver	$y = 4.95x - 878.27$	12	0.90	0.01

VFA refers to volatile fatty acids; IGF-I = Insulin-like growth factor-I; IGFBP-3 = Insulin-like growth factor binding protein-3; GH = Growth hormone; TP = Total protein.

**Table 3.** Relationships between IGF-I (μg/g TP) in liver and IGF-I in tissues, and between IGFBP-3 in liver and IGFBP-3 in tissues (μg/g TP)

Independent (x)	Dependent (y)	Equations	n =	r <sup>2</sup> =	p <
IGF-I					
Liver	Rumen dorsal sac	$y = 1.33x - 88.54$	12	0.95	0.01
Liver	Rumen ventral sac	$y = 0.16x + 45.19$	12	0.94	0.01
Liver	Duodenum	$y = 0.66x + 87.12$	12	0.88	0.01
Liver	<i>Longissimus dorsi</i> muscle	$y = 1.14x + 351.10$	12	0.98	0.01
IGFBP-3					
Liver	Rumen dorsal sac	$y = 1.56x + 0.79$	12	0.85	0.01
Liver	Rumen ventral sac	$y = 0.69x + 0.86$	12	0.96	0.01
Liver	Duodenum	$y = 1.21x - 10.68$	12	0.92	0.01
Liver	<i>Longissimus dorsi</i> muscle	$y = 4.39x + 144.71$	12	0.97	0.01

IGF-I = Insulin-like growth factor-I; IGFBP-3 = Insulin-like growth factor binding protein-3; TP = Total protein.

Significant correlations were also found between the IGF-I in plasma and the IGF-I in the rumen dorsal sac, rumen ventral sac, liver, duodenum and *Longissimus dorsi* muscle (Table 4), and between the IGF-I (y, ng/ml) and the IGFBP-3 (x, ng/ml) in plasma:  $y = 2.98x + 143.51$ ,  $r^2 = 0.94$ ,  $n = 12$ ,  $p < 0.01$ , and between the GH (x, ng/ml) and the IGF-I (y, ng/ml) in plasma:  $y = 67.15x + 39.30$ ,  $r^2 = 0.91$ ,  $n = 12$ ,  $p < 0.01$ .

## DISCUSSION

### Comparison of sheep fed normally and nourished by total intragastric infusions

In normally-fed sheep, dietary carbohydrates can be partly fermented into VFA by rumen microorganisms and VFA is utilized by sheep as the major energy source (Ørskov et al., 1979b; Ørskov and Macleod, 1993). However, it is rather difficult to study the effect of VFA on hormone secretion in normally-fed sheep because of the diurnal variation of VFA concentration in the rumen. The use of the intragastric infusion technique, which could accurately control VFA supply, solved the problem (Ørskov et al., 1979a). It should be noted that there may be some differences in the physiological status between sheep fed normally and those nourished by total intragastric infusions. However, until now there has been no better way than using the intragastric infusion technique to accurately study the effect of VFA on hormone secretion.

### Effect of VFA on IGF-I, IGFBP-3, GH, insulin, glucagon in plasma and IGF-I and IGFBP-3 in different tissues

In rats, higher energy supply increased the abundance of liver IGF-I mRNA and plasma IGF-I, and the liver IGF-I mRNA was positively correlated to the growth of rats (Weller et al., 1994). Restricted energy supply decreased the abundance of IGF-I mRNA in liver and *Longissimus dorsi* muscle of ruminants (Vandehaar et al., 1995), while in humans restricted energy intake decreased the IGF-I and IGFBP-3 in plasma (Smith et al., 1995). In dairy cattle, the IGF-I in plasma was higher in positive energy balance than in negative balance (McGuire et al., 1992). In the present experiment, the IGF-I and IGFBP-3 in plasma and tissues were significantly increased with the VFA infusion level,

which was in agreement with the results of Shen et al. (2004) and Schroeder et al. (2006).

IGF-I is the major hormone that promotes the tissue proliferation and growth in animals (Baldwin, 1999), and the VFA produced in the rumen is used as the major energy source in adult ruminants (Ørskov et al., 1979b; Ørskov and Macleod, 1993). The effects of VFA on IGF-I, IGFBP-3, GH, insulin and glucagon in the present experiment indicated that VFA was not only used as an energy source, but also stimulated the secretion of some hormones. This implies that VFA might improve the development of body tissues and rumen epithelium in growing sheep through stimulating the secretion of some hormones. However, the VFA infused into the rumen in the present experiment was not isocaloric; whether the effects of VFA on hormone secretion resulted from the energy input or from a specific effect of VFA needs to be studied in the future.

In ruminants, many studies indicated that the insulin and glucagon in plasma could be significantly increased by intraruminal infusion of VFA (Bergman et al., 1990), increasing energy supply (Ørskov et al., 1993) or injection of VFA into the thigh vein (Sano et al., 1995). Infusion of sodium butyrate at a pharmacological level to ruminants also increased the insulin in plasma and improved the proliferation of rumen epithelium cells (Neogrady et al., 1989b). In the present experiment, the insulin and glucagon in plasma were significantly increased by VFA infusion which was in agreement with the studies mentioned above.

### Relationships between IGF-I in plasma, IGF-I in liver and IGF-I in different tissues

Most IGF-I is produced in the liver and transported to local tissues through the blood stream, while only a small amount of IGF-I is synthesized in local tissues (McGuire et al., 1992). Therefore, the IGF-I in plasma and in local tissues is mainly dependent upon the IGF-I synthesis in liver. Indeed, in the present experiment, significant positive correlations were found between the IGF-I in liver and in different tissues, and between the IGF-I in plasma and the IGF-I in liver and other tissues with high correlation coefficients. These results were in agreement with those of Cordano et al. (2000), who reported that the IGF-I mRNA in the liver of heifers, beef calves, fattening bullocks and

**Table 4.** Relationships between IGF-I in plasma (ng/ml) and IGF-I in tissues ( $\mu\text{g/g}$  TP)

Independent (x)	Dependent (y)	Equations	n =	$r^2 =$	p <
Plasma	Rumen dorsal sac	$y = 4.70x - 788.08$	12	0.93	0.01
Plasma	Rumen ventral sac	$y = 0.56x - 37.58$	12	0.91	0.01
Plasma	Liver	$y = 3.47x - 511.17$	12	0.94	0.01
Plasma	Duodenum	$y = 2.34x - 263.27$	12	0.90	0.01
Plasma	<i>Longissimus dorsi</i> muscle	$y = 4.01x - 244.86$	12	0.96	0.01

IGF-I = Insulin-like growth factor-I; TP = Total protein.

adult bulls was positively correlated with the IGF-I in plasma ( $r = 0.92$ ). It could be speculated that the IGF-I in plasma is a suitable parameter to represent the IGF-I status in sheep and it may be unnecessary to do slaughter trials for the analysis of IGF-I in liver and other tissues.

#### Relationships between hormones in plasma

The IGFBP-3 in plasma is the carrier for IGF-I transportation in blood which is also a pool of IGF-I to maintain relatively stable concentrations of IGF-I in healthy animals (Simon et al., 1992). Some studies indicated that the IGF-I in plasma and IGF-I mRNA abundance in liver could be increased by GH (Coleman et al., 1994; Kobayashi et al., 1995). Also, the IGFBP-3 and insulin in plasma of dairy cattle and sows could be increased by GH treatment (Coleman et al., 1991; Cohick et al., 1992). The results in the present experiment showed that in growing sheep the IGF-I in plasma was significantly related to and influenced by the GH in plasma.

#### CONCLUSION

The IGF-I, IGFBP-3, GH, insulin and glucagon in plasma and the IGF-I and IGFBP-3 in rumen dorsal sac, rumen ventral sac, liver or *Longissimus dorsi* muscle of growing sheep sustained by total intragastric infusions were significantly increased with the level of ruminal infusion of mixed VFA. Significant correlations were found between the VFA infusion level and the IGF-I and IGFBP-3 concentrations in plasma.

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