Asian-Aust. J. Anim. Sci.
Vol. 24, No. 10 : 1393 - 1398
October 2011

www.ajas.info
http://dx.doi.org/10.5713/ajas.2011.11044

Relationships between Methionine Supply, Nitrogen Retention and Plasma Insulin-like Growth Factor-I in Growing Sheep Nourished by Total Intragastric Infusions

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ABSTRACT : Four 4-month old Charolais×Dorset male sheep (initial liveweight 25.0±1.1 kg), fitted with rumen and abomasal fistulas and nourished by total intragastric infusions, were used to study the relationships between methionine (Met) supply, nitrogen (N) retention and plasma insulin-like growth factor-I (IGF-I). Four graded levels of Met, i.e. 0 g/16 g N, 1.76 g/16 g N, 3.52 g/16 g N and 7.04 g/16 g N, were infused into abomasums as experimental treatments. The sheep and treatments were allocated in a 4×3 incomplete Latin square design (Yudon square design). The experiment lasted 3 periods and each period was 10 days. Quadratic correlations were found between Met level (x, g/16 g N) and N retention (y, g/d): $y = -0.03x^2+0.41x+2.62$, $r^2 = 0.66$, n = 12, p = 0.008, and between methionine level (x, g/16 g N) and plasma IGF-I concentration (y, ng/ml): $y = 0.80x^2-4.53x+190.24$, $r^2 = 0.51$, n = 12, p = 0.009. No significant correlation was found between plasma IGF-I (x, ng/ml) and N retention (y, g/d) (p>0.05). It was concluded that Met level had a significant influence on N retention and plasma IGF-I concentration whereas IGF-I might not be an important mediator in the regulation of N metabolism by Met in growing sheep nourished by total intragastric infusions. (**Key Words :** Methionine, Nitrogen Retention, IGF-I, Sheep)

INTRODUCTION

As the first limiting amino acid (AA), methionine (Met) is important for the growth, production and N utilization in ruminants. Lynch et al. (1991) reported that supplementing 0.28% encapsulated lysine (Lys) plus 0.11% encapsulated Met which contained 1.3% active Lys and 1.1% active Met increased N retention in lamb-nursing-ewes. Schelling et al. (1973) reported that increments of Met infusion from 0 g to 3 g/d to sheep fed a high quality diet containing 14.1% protein also resulted in increased N retention. Sun et al. (2007) reported that supplementing 0.77 g ruminally-protected Lys and 0.91 g ruminally-protected Met/100 g of concentrates (dry matter basis) to growing goats increased N retention and plasma essential AA concentration and reduced ruminal NH_3 and plasma urea N concentrations. Goulas et al. (2003) found that the use of ruminally-protected Met combined with animal fat, in diets of high producing ewes, significantly increased milk yield at the first stage of lactation. Misciattelli et al. (2003) and Cho et al. (2007) also found that supplementing ruminally-protected Met increased milk fat and milk protein of dairy cows.

Some hormones such as insulin-like growth factor-I (IGF-I), growth hormone (GH), and insulin play important roles in the regulation of protein metabolism while nutritional status could affect circulating levels of these plasma hormones. Pell et al. (1993) reported that total hepatic IGF-I messenger RNA (mRNA) and total IGF-I mRNA transcripts in muscle of sheep were significantly increased by GH, protein, and energy status. Lee et al. (2005) found that feeding high protein diets (1.38 kg crude protein/d) to Holstein steers significantly increased plasma IGF-I concentration over those fed low protein diets (0.66 kg crude protein/d). As the limiting amino acids, Lys and Met may play important roles in regulating circulating levels of some plasma hormones. Stubbs et al. (2002) found in cells cultured with a Met supply limited to 0.2 times that of the physiological concentration of Met in sheep plasma

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Received February 20, 2011; Accepted May 11, 2011

had inhibited IGF-I RNA expression. Fernández-Figares et al. (2007) found that serum insulin and IGF-I concentrations were decreased when Lys deficient diets were fed to pigs while GH remained unaffected. Roy and Lapierre (2000) and Ren et al. (2007) found in pigs that plasma insulin concentration was increased by dietary Lys, while plasma IGF-I and GH concentrations were not affected. From the results reviewed above, it could be speculated that Met level would affect the concentrations of some plasma hormones and consequently regulating the nitrogen metabolism in sheep. The objective of the experiment was to study the relationships between Met supply, N retention and plasma IGF-I, GH, insulin and other parameters in growing sheep nourished by total intragastric infusions.

MATERIALS AND METHODS

Animals

Four 4-month old Charolais×Dorset male sheep, with an average liveweight of 25.0±1.1 kg, fitted with rumen and abomasal fistulas, were used in the experiment.

Experimental design

The animals were assigned in a 4×3 incomplete Latin square design (Yudon square design). The AA mixture was used as the N source and a total of 1.43 times maintenance N requirement was supplied, with the assumption that the maintenance N was 350 mg kg^{-1} $W^{0.75}$ d^{-1} (MacLeod et al., 1982). The AA proportion of the mixture was calculated based on the optimum essential AA composition (Fraser et al., 1991). Four graded levels of Met, i.e. 0 g/16 g N, 1.76 g/16 g N, 3.52 g/16 g N and 7.04 g/16 g N, were used in the AA mixture as experimental treatments. Glycin was used to balance the N level of each treatment (Table 1). The total energy supply was 1.20 times the maintenance energy requirement, with the assumption that the maintenance energy was 450 KJ kg^{-1} $W^{0.75}$ d^{-1} (MacLeod et al., 1982). The total energy was derived from four sources: mixed VFA (the molar proportion of acetic, propionic and butyric acids was 65:25:10) 0.74 M, glucose 0.15 M, corn oil 0.15 M and AA mixture 0.16 M. Glucose and corn oil were used to decrease the VFA infusion level required to maintain the rumen pH in a normal range as well as to supply glucose and long chain fatty acids to the sheep.

Table 1. Composition of mixed amino acids (g AA/16 g N)

Amino acids	Met levels			
	0	1.76	3.52	7.04
Arg	4.07	4.07	4.07	4.07
His	3.41	3.41	3.41	3.41
Isoleu	5.81	5.81	5.81	5.81
Leu	10.50	10.50	10.50	10.50
Lys	9.54	9.54	9.54	9.54
Cys	0.69	0.69	0.69	0.69
Phe	5.19	5.19	5.19	5.19
Tyr	5.43	5.43	5.43	5.43
Thr	4.51	4.51	4.51	4.51
Val	6.94	6.94	6.94	6.94
Try	1.46	1.46	1.46	1.46
Met	0.00	1.76	3.52	7.04
Gly	3.56	2.66	1.78	0.00

Preparation of stock solutions

The VFA stock solution, buffer stock solution, vitamins mixture, trace elements stock solution, and mineral stock solution were prepared according to MacLeod et al. (1982). The AA mixture solution was made up using distilled water. The N content of AA mixture stock solution was 0.0122 g N/g (Table 2). Glucose and corn oil were blended in the AA mixture according to Zhao et al. (1995).

Calculation and dilution of the stock solution

The amount of the stock solutions and mixtures infused was calculated based on the nutrient requirements of the animals and the experimental design (Table 3). Distilled water was used to dilute the stock solutions and mixtures. The final diluted weight of all infusates was $W^{0.75}$×5.3 kg, of which the VFA solution was diluted to yield the final weight to be $W^{0.75}$×1.325 kg, buffer $W^{0.75}$×2.65 kg (Sun and Zhao, 2009), and the mixture of AA, minerals, vitamins and trace elements $W^{0.75}$×1.325 kg.

Infusion procedure

The sheep were housed in individual metabolism cages. Two multi-channel peristaltic pumps were used for infusion. Two catheters were connected to the rumen fistula of each sheep to infuse the buffer and VFA solutions, respectively. One catheter was connected to the abomasal fistula of each sheep to infuse the AA mixture (including vitamins, trace elements, glucose and corn oil). The infusion continued from 8:30 am to 8:30 pm every day during each 10 day infusion period, of which the first 7 days were for pre-infusion and the last 3 days for sample collection. During the infusion period, no feed but drinking water was supplied to the sheep.

Measurement and sampling

The sheep were weighed in the morning on the first day and the last day of the infusion trial and the weight of the sheep was recorded. During the last 3 days of each infusion period the urine and the faeces were completely collected and 5% of the urine and all the faeces were sampled for later analysis. At 10:00 in the morning on the last day of each infusion period a 15 ml blood sample was taken from the jugular vein into a vacuette (Greiner Bio-one Company, the anticoagulant was K_3EDTA). All the samples were kept in a freezer at -20°C.

Table 2. Components of stock solutions and mixtures

Item	Components	Concentration (g/10,000 g)	Notes
VFA stock solution	Acetic acid	3,922.86	Purity 99.5%
	Propionic acid	1,870.71	Purity 99.0%
	Butyric acid	899.08	Purity 98.0%
	$CaCO_3$	180.00	Purity 99.99%
	Water	3,127.35	Distilled water
Buffer stock solution	$NaHCO_3$	733.67	Purity 99.5%
	$KHCO_3$	381.91	Purity 99.5%
	NaCl	70.35	Purity 99.5%
	Water	8,814.07	Distilled water
Mixed amino acids stock solution	Arg	31.03	Purity 99.0%
	His	26.00	Purity 99.0%
	Isoleu	44.30	Purity 99.0%
	Leu	80.06	Purity 99.0%
	Lys	72.74	Purity 99.0%
	Met	26.84	Purity 99.0%
	Cys	5.26	Purity 99.0%
	Phe	39.57	Purity 99.0%
	Tyr	41.40	Purity 99.0%
	Thr	34.39	Purity 99.0%
	Val	52.92	Purity 99.0%
	Try	11.13	Purity 99.0%
	Na_2CO_3	54.00	Purity 99.5%
	Vitamin mixture	256.00	Prepared in advance
	Water	9,224.34	Distilled water
Vitamin mixture	Thiamine hydrochloride	5.16	Purity 99.0%
	Riboflavine	4.13	Purity 99.0%
	Nicotinic acids	4.13	Purity 99.0%
	Choline chloride	851.77	Purity 99.0%
	Pyridoxine hydrochloride	2.06	Purity 99.0%
	p-amino-benzoic acid	0.10	Purity 99.0%
	Calcium DL-pentothenate	6.50	Purity 99.0%
	Folic acid	0.21	Purity 99.0%
	Cyanocobalamin	0.03	Purity 99.0%
	Myo-inositol	154.87	Purity 99.0%
	D-biotin	0.62	Purity 99.0%
	2-Methy-1,4-Napthaquinone	0.52	Purity 99.0%
	DL-a-tocopherol acetate	10.32	Purity 99.0%
	Ethanol	813.57	Purity 99.0%
	Fish liver oil	1,951.32	Purity 99.99%
	Water	6,194.68	Distilled water
Mineral stock solution	$Ca(H_2PO_4)\cdot H_2O$	163.04	Purity 92.0%
	$MgCl_2\cdot 6H_2O$	76.53	Purity 98.0%
	Water	9,760.43	Distilled water
Trace minerals stock solution	$FeSO_4\cdot 7H_2O$	210.10	Purity 99.0%
	$ZnSO_4\cdot 7H_2O$	12.26	Purity 99.5%
	KI	11.27	Purity 98.5%
	$MnSO_4\cdot H_2O$	4.44	Purity 99.0%
	$CuSO_4\cdot H_2O$	2.53	Purity 99.0%
	$CoSO_4\cdot 7H_2O$	2.21	Purity 99.0%
	NaF	8.06	Purity 98.0%
	Water	9,749.13	Distilled water

Table 3. Effect of Met infusion level on N digestion and metabolism

Parameters	Met levels (g AA/16 g N)				SEM	p	p	
	0	1.76	3.52	7.04			L	Q
Initial live weight (kg)	23.45±0.95	23.61±2.52	23.77±2.66	23.87±1.66	1.86	0.925	0.848	0.668
Body weight loss (kg/d)	2.29±0.64	2.80±0.30	1.73±0.41	2.02±0.52	0.42	0.877	0.675	0.916
N intake (mg/d)	5,512±123	5,564±212	5,622±199	5,644±164	159	0.919	0.872	0.761
Faecal N (mg/d)	362±235	300±195	318±126	270±154	164	0.936	0.814	0.552
Apparent N digestibility (%)	93.4±4.4	94.5±3.6	94.3±2.5	95.3±2.6	3.0	0.918	0.497	0.800
Urinary N (mg/d)	2,649±229^{A}	1,704±59^{BC}	1,844±254^{B}	1,314±192^{C}	301	0.001	0.001	0.002
N retention (mg/d)	2,501±568^{A}	3,560±438^{B}	3,458±220^{B}	4,061±245^{C}	439	0.008	0.003	0.008
Efficiency of N utilization (%)	45.2±9.2^{A}	60.4±0.6^{B}	64.9±5.1^{B}	71.9±3.4^{B}	7.0	0.003	0.002	0.005

Data in the same row labeled with different lowercase superscripts mean significant difference ($p<0.05$), different capital superscripts mean extremely significant difference ($p<0.01$).

Determination and analysis

The total N of faeces and urine were determined using the Kjeldahl method. Plasma insulin was analyzed using specific radioimmunoassay kits (Beijing Sino-UK Institute of Biological Technology). Plasma IGF-I was analyzed using RIA using specific radioimmunoassay kit (Beijing Sino-UK Institute of Biological Technology). Plasma glucose was analyzed using GOD/POD enzymatic method using commercial kit (Beijing Sino-UK Institute of Biological Technology). Plasma urea nitrogen (PUN) was analyzed using OPA colorimetric method (Beijing Sino-UK Institute of Biological Technology).

Calculation and statistical analysis

The results are presented as mean±SEM (standard error of mean).

N retention (mg/d)
= N supply (mg/d)-Faecal N (mg/d)-Urinary N (mg/d)

Efficiency of N utilization (%)
= N retention (mg/d)/N supply (mg/d)×100

Comparison of the differences of parameters between treatments and the correlations between Met level, N retention and plasma concentrations of hormones were analyzed using SAS (version 8.0).

RESULTS

As the infusion continued, the sheep gradually stopped ruminating and hardly drank any water, urine excretion markedly increased and less and less feed particles were found in the rumen fluid. The liveweight of the sheep decreased about 2 kg during the three infusion periods.

The results of N metabolism are shown in Table 3. No differences were found in faecal N excretion or apparent N digestibility between different treatments ($p>0.05$). Urinary N significantly decreased and the N utilization efficiency was increased with Met infusion level. It was found that N retention (y, g/d) increased with Met level (x, g/16 g N) in a quadratic manner: $y = -0.03x^2+0.41x+2.62$, $r^2 = 0.66$, $n = 12$, $r^2 = 0.66$, $p = 0.008$, Figure 1.

The concentrations of hormones, PUN and glucose in plasma are shown in Table 4. No significant differences were found in GH, insulin, PUN and glucose concentration between different treatments ($p>0.05$) while IGF-I concentration of treatment 7.04 g Met/16 g N was significantly higher than that of other treatments. A quadratic correlation was found between Met infusion level (x, g/16 g N) and plasma IGF-I concentration (y, ng/ml): $y = 0.80x^2-4.53x+190.24$, $n = 12$, $r^2 = 0.51$, $p = 0.009$, Figure 2.

DISCUSSION

Liveweight loss

Although 1.2 times maintenance energy and 1.4 times maintenance N were supplied to the sheep, the sheep lost

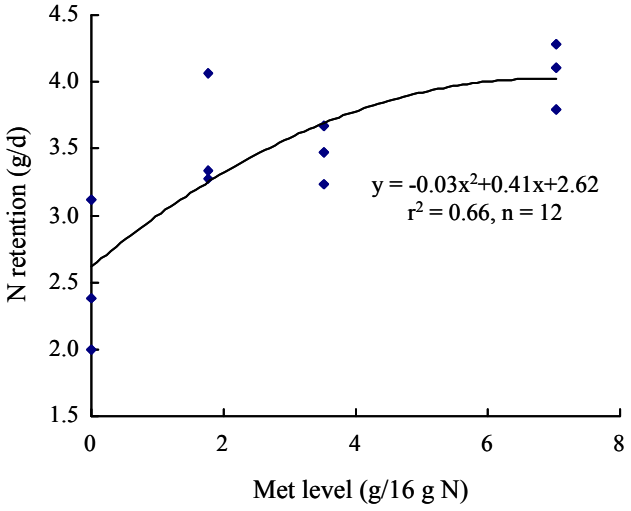

Figure 1. Relationship between Met level and N retention.

Table 4. Effect of Met infusion level on Plasma hormones, PUN and plasma glucose concentration

Parameters	Met levels (g AA/16 g N)				SEM	p	p	
	0	1.76	3.52	7.04			L	Q
GH (ng/ml)	2.81±0.61	3.01±0.50	3.30±1.03	3.35±0.44	0.63	0.754	0.293	0.540
INS (uIU/ml)	16.71±5.36	15.51±2.68	13.04±2.40	16.69±4.53	3.71	0.647	0.992	0.481
IGF-I (ng/ml)	191.27±8.74^{a}	182.00±3.61^{a}	186.27±4.72^{a}	197.71±6.87^{b}	8.49	0.016	0.627	0.009
PUN (mmol/L)	2.04±0.25	1.70±0.13	2.13±0.09	2.07±0.08	0.12	0.854	0.453	0.450
Glucose (mmol/L)	6.25±0.61	5.44±0.53	5.65±0.10	6.05±0.30	0.47	0.757	0.892	0.561

Data in the same row labeled with different lowercase superscripts mean significant difference ($p<0.05$), different capital superscripts mean extremely significant difference ($p<0.01$).

about 2 kg liveweight during the infusion trial. Since no feedstuffs were given to the sheep after the infusion started and some faeces were voided by the sheep, the liveweight loss must have been due to the loss of the digestive contents from the sheep. The results are in agreement with Sun and Zhao (2009) who reported that some faeces were voided by the sheep nourished by intragastric infusions.

N metabolism

In growing goats, Sun et al. (2007) found that supplementing ruminally-protected Met and Lys significantly decreased urinary N and increased N retention. In dairy cows, Cho et al. (2007) found that supplementing ruminally-protected Met decreased urinary N and increased N retention. In the present experiment, N retention and N utilization efficiency were increased and urinary N was decreased with Met infusion level. The results were in accordance with the results mentioned above.

It was found that when the Met level increased from 0 g/16 g N to 1.76 g/16 g N, the N utilization efficiency was significantly increased whereas when the Met level increased from 1.76 g/16 g N to 3.52 g/16 g N and 7.04 g/16 g N, the N utilization efficiency was not significantly changed. The results were in agreement with Fraser et al. (1991) who reported that the optimum proportion of Met was 1.76 g/16 g N which was calculated based on the optimum essential AA composition of ruminant. The results indicated that an excessive Met supply above the Met requirement would not improve the N utilization efficiency in sheep. The quadratic correlation between Met level and N retention clearly indicated the trend.

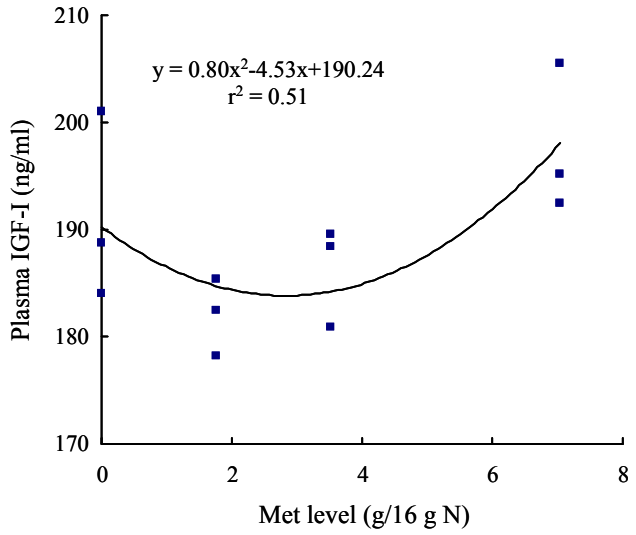

Figure 2. Relationship between Met level and plasma IGF-I.

Plasma parameters

Stubbs et al. (2002) found that in cell culture Met is the key limiting AA involved in the modulation of IGF-I expression in the ovine liver. Miller et al. (2005) found that reduction of dietary Met content from 0.43% to 0.15% reduced serum IGF-I and insulin levels in the mouse. Oliver et al. (1993) reported that plasma IGF-I concentration was not regulated by an AA mixture infused into fetal sheep. Misciattelli et al. (2003) reported that supplementation of 24 g of ruminally-protected Lys or 12 g of ruminally-protected Met to dairy cows did not affect plasma IGF-I. In the present study, it was found that plasma IGF-I concentration and N retention were both significantly increased with Met infusion level, indicating that a balanced AA proportion may enhance IGF-I secretion and consequently increased protein anabolism, but the influence might depend on the level of Met supplied.

It is widely believed that GH could accelerate the growth of animals. In the present study, it was found that plasma GH concentration was not affected by the Met level even though Met infusion increased N retention in the sheep. The results were in agreement with Zakin et al. (1975), who reported that the modification of Met did not greatly modify the immunological reactivity of human and horse GH.

Misciattelli et al. (2003) reported that supplementation of 24 g of ruminally-protected Lys or 12 g of ruminally-protected Met to dairy cows did not affect plasma insulin, glucose and urea concentrations. Berthiaume et al. (2001) reported that feeding 72 g of ruminally-protected Met daily

to dairy cows resulted in higher concentrations of urea-N and glucose in arterial plasma; whereas Preynat et al. (2009) found that supplementation of 18 g ruminally-protected Met to dairy cows did not affect the whole-body glucose flux. In the present study, no significant differences were found in plasma insulin, glucose and PUN concentrations between different treatments. The results indicated that differences between different studies might result from the level of supplemented Met. Further research is needed to study the influence of Met level on plasma glucose and PUN in sheep.

CONCLUSION

Met supply significantly influenced the N retention and plasma IGF-I concentration in growing sheep in quadratic manners, whereas no significant relationship between Met level and plasma IGF-I was found, indicating that plasma IGF-I might not be an important mediator in regulating N metabolism by Met.

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