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Effects of Intra-duodenal Infusion of Limiting Amino Acids on Plasma Insulin-like Growth Factor I, Growth Hormone, Insulin and Liver Insulin-like Growth Factor I mRNA in Growing Goat Fed a Maize Stover-based Diet

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ABSTRACT : The effects of intra-duodenal infusion of methionine (Met), lysine (Lys) and leucine (Leu) on dry matter intake (DMI), the concentrations of insulin-like growth factor I (IGF-I), growth hormone (GH) and insulin in plasma, and liver IGF-I mRNA level were investigated in two experiments for Liuyang Black growing wether goats. In Experiment 1, three goats (10.0±0.1 kg) were fitted with ruminal, proximal duodenal and terminal ileal fistulaes to determine the infusion amounts of Met, Lys and Leu at the duodenum according to essential amino acid flows into the duodenum and their apparent digestibility. The infusion amounts were 0.77 g/d, 0.91 g/d and 0.58 g/d respectively. In Experiment 2, 4 groups of goats (10.0±0.2 kg) for each group, were cannulated at the duodenum, and were infused with a mixture of Met, Lys and Leu (Control), or mixtures with 21% Met, Lys or Leu replaced with glutamate respectively on a nitrogenous basis. The replacement of 21% Met, Lys or Leu with glutamate did not affect intakes of maize stover, concentrate or both ($p>0.05$) when compared with the control. The replacement of 21% Met or Lys significantly ($p<0.05$) reduced plasma GH, insulin and IGF-I concentrations and liver IGF-I mRNA level. The replacement of 21% Leu with glutamate reduced ($p<0.05$) plasma IGF-I concentration only, but not plasma insulin and GH, as well as liver IGF-I mRNA level ($p>0.05$). The close relationships between supplying Met and Lys in the lumen of the duodenum and plasma IGF-I, GH and insulin concentrations, as well as liver IGF-I mRNA level in this study indicate that the effects of the limiting amino acids on nutrition of animals are likely intermediated via their effects on these hormones, and these hormone profiles could be used as intermediate markers for the limiting order of amino acids. (**Key Words :** Limiting Amino Acids, GH, IGF-I, Insulin, IGF-I mRNA)

INTRODUCTION

The concept of the dietary ideal protein that is widely accepted in poultry and pig production (Chung and Baker, 1992; Li et al., 2003) has led to growing interest in studying limiting amino acids (optimal amino acid pattern) at the duodenum for optimizing nutrition of the small intestine in ruminants (Klemesrud et al., 1997; Abe et al., 1998; Wang and Lu, 2002). Limiting amino acids and their order depend upon animal species, breeds, and diets. For example, Lys is the first limiting amino acid to lactating cows fed with a corn-based diet (Schwab et al., 1992), whereas Arginine (Arg), Met, and Leu are the first, second and third limiting amino acids respectively to growing cattle fed wheat silage (Rayland-Gray et al., 1997).

Insulin-like growth factor I, GH and insulin play a key role in growth of animals (Spicer et al., 1993; Georgieva et al., 2003). Some studies have demonstrated that dietary essential amino acids could influence plasma IGF-I, GH and insulin concentrations (Bolze et al., 1985; Rayland-Gray et al., 1997; Yang et al., 2000) and liver IGF-I mRNA level in animals (Harp et al., 1991; Stubbs et al., 2002). However, there are few conducted experiments to compare the affecting extent of amino acids limiting order on plasma IGF-I, GH, insulin concentrations and liver IGF-I mRNA level in previous studies. Therefore, this experiment was carried out to investigate the effects of respectively removing same proportion of the first, second and third limiting amino acids from the duodenal infused amino acids mixtures on the plasma IGF-I, GH, insulin concentrations and liver IGF-I mRNA level in growing goats.

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Table 1. The dietary ingredients and chemical composition [a]

Ingredient	%	Composition (%)	
Corn	22.5	ME [b] (MJ/kg)	9.45
Wheat bran	18.0	Crude protein	13.8
Soybean meal	6.2	Neutral detergent fiber	38.0
Maize stover	50.0	Acid detergent fiber	26.0
Urea	0.7	Calcium	0.27
Salt	0.6	Phosphorus	0.34
Premix [c]	2.0		

[a] Values, expressed on a DM basis, are the average of two replicates.
[b] Metabolizable energy values were reported by Zhang and Zhang (1998), and the others were determined values.
[c] Premix contained per kilogram: 119 g $MgSO_4 \cdot H_2O$, 2.5 g $FeSO_4 \cdot 7H_2O$, 0.8 g $CuSO_4 \cdot 5H_2O$, 3 g $MnSO_4 \cdot H_2O$, 5 g $ZnSO_4 \cdot H_2O$, 10 mg Na_2SeO_3, 40 mg KI, 30 mg $CoCl_2 \cdot 6H_2O$, 95,000 IU vitamin A, 17,500 IU vitamin D, and 18,000 IU vitamin E.

MATERIALA AND METHODS

Two experiments were conducted using Liuyang Black goats (a local breed in China for meat production, with mature body weight about 20 kg). The experimental procedures were approved by the Animal Care Committee, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, China.

Experiment 1

This experiment was carried out to determine the amounts of Met, Lys and Leu to be infused into the lumen of the duodenum, which was used as the basic data to design Experiment 2. Three 4-month-old growing wether goats, initially weighed at 10.0±0.1 kg, were kept individually in stainless metabolism cages in a temperature-controlled (at 21°C) and constant-lighted house. They were surgically fitted with the ruminal, proximal duodenal and terminal ileal fistulae. Two weeks were allowed for recovery from the surgery. During this period the wound was cleaned with potassium iodide and then covered with the anti-inflammatory power, and penicillin K (80,000 IU/head) was administered twice daily to control infection. All goats were allowed freely to fresh water and were fed equal amounts of ground maize stover (1 cm length, to 5% refusal) and concentrate (see Table 1) at 0700 and 1900 to meet 1.3 times maintenance requirements of metabolizable energy according to the nutrient requirements of Chinese goats (Lu et al., 1996). The experimental period was 14 d in length with 7 d adaptation and 7 d sample collection. During the sample collection period (d 8 to d 14), 1 g of Cr_2O_3 as the particle-phase digesta marker was administered via the rumen cannula at 0600, 1200, 1800 and 2400 h respectively. From d 12 to d 14, duodenal and ileal digesta samples were taken at 6 h intervals. The sampling time was changeable among collecting days in order to collect representative digesta samples, and the actual times were 0100, 0700, 1300, 1900 on d 12; 0300, 0900, 1500, 2100 on d 13; and 0500, 1100, 1700, 2300 on d 14. The duodenal and ileal digesta were collected by natural flow into plastic collection bags attached to the cannulas with cable ties, and digesta pH was adjusted to 3.5 using 9 mol/L H_2SO_4. Digesta was fozen daily at -20°C. Digesta samples from each collecting time were freeze-dried, pooled for each goat, and ground in a laboratory mill (DF-2, Changsha Instrument Factory, China) through a 1-mm screen, and mixed before analysis. The samples of the diets were ground similarly.

The duodenal and ileal digestibilities of amino acids were calculated according to the following equations as described by Lu and Xie (1991):

$$Q_d = C_t/C_d$$
$$Q_i = C_t/C_i$$
$$DFAA_i = CDAA_i \times Q_d$$
$$IFAA_i = CIAA_i \times Q_i$$
$$DAA_i = (DFAA_i - IFAA_i)/DFAA_i \times 100\%$$

Where Q_d and Q_i are DM flow (g/d) into the duodenum and the ileum respectively, C_t is the total amount of Cr_2O_3 administrated into the rumen per d, C_d and C_i stand for Cr_2O_3 concentrations in the dried duodenal and ileal digesta respectively, $DFAA_i$ and $IFAA_i$ are AAi flows at the duodenum and the ileum, $CDAA_i$ and $CIAA_i$ are AAi concentrations in the dried duodenal and ileal digesta, and DAA_i stands for ileal apparent digestibility of AAi.

The amounts of Met, Lys and Leu infused into the lumen of the duodenum were calculated using the following equation as described by Shan and Tan (2004):

$$\frac{DAAi}{Ri}\Delta Xi - \sum_{i=1}^{n} DAAi \times \Delta Xi = DtQt - \frac{Di \times DFAAi}{Ri}$$

Where D_t is the ileal digestibility of total amino acids, Q_t is the duodenal flow of total amino acids, ΔX_i is the calculated amount of AA_i that should be infused into the duodenum, and R_i is the AA_i proportion to total amino acids in the muscle (Shan and Tan, 2004). According to the linear-equation matrix using the basic computing language software, ΔX_i for each amino acid was calculated out. If $\Delta X_i \geq 0$, it represents a state that the AA_i should be supplemented into the duodenum to balance the muscle amino acid pattern. Considering the objective of this study, nine essential amino acids except for non-essential amino acids were evaluated using the above model to calculate the infusion amount (see Table 2).

Experiment 2

Twelve 4-month-old growing wether goats, initially weighed at 10.0±0.2 kg, were housed as in Experiment 1.

Table 2. Flows (g/d) of essential amino acids through the duodenum and ileum as well as their apparent digestibility (%) in the small intestine of growing goats in Experiment 1

AA	Duodenal flow	Ileal flow	Intestinal digestibility	Measured amino acid profile (% to total amino acids) in muscle[b]
His	2.06 (0.25)[a]	0.74 (0.12)	64.2 (3.95)	5.9
Arg	1.54 (0.06)	0.29 (0.06)	81.0 (4.96)	16.7
Thr	1.54 (0.21)	0.71 (0.15)	54.6 (5.26)	11.4
Val	1.94 (0.26)	0.72 (0.15)	63.8 (3.67)	11.2
Met	0.12 (0.02)	0.08 (0.02)	43.4 (8.82)	4.1
Lys	1.56 (0.15)	0.60 (0.14)	61.9 (7.13)	16.0
Ile	1.24 (0.18)	0.45 (0.09)	64.4 (3.68)	8.7
Leu	2.09 (0.32)	0.73 (0.15)	65.2 (3.94)	17.1
Phe	1.91 (0.36)	0.56 (0.11)	70.6 (1.86)	8.9

[a] Numerical values in the parentheses are the standard error of means.
[b] From Shan and Tan (2004). The measured amino acid profile in LD muscle was provided, which was used for calculation of supplementation of Met, Lys and Leu.

The goats were housed and managed at the same conditions as in Experiment 1. The feeds were offered to provide the same plane of nutrition (approximately 1.3 folds of the energy maintenance) to each individual. The animals were cannulated at the duodenum according the same surgical procedure as in Experiment 1 in order to infuse amino acids into the duodenum. After the recovery from the surgery, the animals were randomly assigned to four treatments, with 3 animals per treatment. The control group was infused with a mixture of Met, Lys and Leu into the duodenum, whereas the other three groups were infused with the amino acid mixture with 21% lesser Met (treatment A), Lys (treatment B), and Leu (treatment C) respectively compared with that for control group. The reduced Met, Lys or Leu was replaced with L-GluNa (Rianxi monosodium glutamate, Inc, China) to ensure same nitrogen (N) level for all treatments. Before infusion, the mixture of Met, Lys and Leu was dissolved with 500 ml distilled water, the pH of amino acids solution was adjusted to 6.50 by 4 M HCl, and the adjusted solution was put into a bottle. The bottle, filled with the amino acid solution, was hung over the metabolism cage, and connected with the duodenal fistula through one-off perfusion tube. The flow rate of the solution was controlled at 0.58 ml/min. The infusion lasted for 7 d, prior to the amino acid infusion, an equivalent volume of distilled water was infused into the duodenum for 3 d as an adaptation period. Feed intake for each animal was recorded daily from d 1 to d 7.

The amino acids infusion was terminated in the morning of d 8. Ten ml of blood sample was taken from the jugular vein. The goats were immediately anesthetized, the abdominal cavity was opened, and liver sample (about 2 g) was taken. The liver sample was immediately frozen in liquid N and stored at -70°C.

The blood sample for analyzing plasma IGF-I, GH, and insulin were treated with sodium heparin, and plasma was harvested by centrifugation at 3,000×g for 20 min. The plasma was stored at -20°C until the analysis.

Analytical procedures

Digesta samples were analyzed for DM and Kjeldahl N content using the methods of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber and ADF were determined using the procedure of Van Soest et al. (1991). For amino acid analysis, except for Met and cysteine (Cys), approximately 0.1 g of sample was weighed out into screw-capped test tubes and mixed with 3 ml of 6 N HC1. The tubes were flushed with N and then heated in an oven at 110°C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and centrifuged at 1,110×g for 15 min at room temperature. The samples were analyzed according to the method of Jones and Gilligan (1983) using a Varian 5000 HPLC system (Varian Associates, Sunnyvale, CAj with a reversed-phase column previously described by Dugan et al. (1989). The amino acids in the sample were derivatized with an o-phthaldialdehyde reagent solution and detected spectrofluorometrically. Met and Cys were determined as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid; the oxidation procedure was carried out according to AOAC (1984). The oxidized samples were dried according to the procedure described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the samples that were not oxidized. Chromium content of duodenal and ileal samples were determined as described by Williams et al. (1962). Duplicate feed samples were used for the chemical analysis and values were reported on a DM basis.

The concentrations of plasma IGF-I, insulin and GH were determined by RIA kits for goats (The Institute of Shanghai Biological Manufactured Product, China) using γ-radioimmunocounter (USTC Chuangxin Co., Ltd. Zonksa. Branch). Before IGF-I assay, plasma samples were

Table 3. Effects of amino acids mixture infusion (control) and 21% replacement of individual methionine (-Met), lysine (-Lys) or leucine (-Leu) with glutamate at the duodenum on feed intake of growing goats fed a maize stover-based diet

	-Met	-Lys	-Leu	Control	SEM
Maize stover (g/d)	173 [a]	168 [a]	159 [a]	154 [a]	7.5
Concectrate (g/d)	168[a]	168 [a]	148 [a]	176 [a]	6.6
Total DMI (g/d)	342[a]	321[a]	308[a]	313[a]	12.5

[a, b, c] Values with the same letter in a row show that the difference between two group is not significant ($p>0.05$), with adjacent letters different shows that the difference between two group is significant ($p<0.05$), with interphase letter shows that the difference between two group is very significant ($p<0.01$).

extracted using the procedure of Bruce et al. (1991) to remove IGF-I binding protein. The intra- and interassay coefficient of variability (CV) for IGF-I and insulin were 4.6 and 8.9%, the intra- and interassay CV for insulin were 8.2 and 12.4%, and the intra- and interassay CV for GH were 8.4% and 10.3%.

IGF-I gene (961-bp, accession number D11378), and glyceraldehydes-3-phosphate (GAPDH) gene (541-bp, accession number AJ 431207). The concentration of GAPDH expression in liver tissue is almost kept stablely under normally physiological status, it can be thought that GAPDH expression is not affected by removing same proportion of amino acids from limiting amino acids mixture, whereas IGF-I expression will be affected, so GAPDH is treated as a internal stand gene when studying the affecting extent of intra-duodenal infusion of limiting amino acids on liver insulin-like growth factor I mRNA in growing goat) were searched from GeneBank (http://www.ncbi.nlm.nih.gov). Primers of IGF-I and GAPDH genes were designed by DNAMAN 4.15 design primer software (Lynnon Biosoft, Canada). Primers for goat IGF-I gene (Forword 5'-GATGC TCTCC AGTTC GTGT-3', reverse 5' TGAGA GGCAG GGACT AAGA-3') and goat GAPDH gene (5'-GGGTG ATGCT GGTGC TGAGT-3', reverse 5'-TCCCT CCACG ATGCC AAA-3') were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co Ltd (Shanghai, China).

Total RNA was extracted from the liver sample using UNIQ-10 pillar Trizol total RNA extraction kit (Shanghai Sangon Biological Engineering Technology & Service Co. Ltd, China). The A_{260}/A_{280} for total RNA was between 1.8 and 2.0.

Total RNA from the sample was reversely transcribed into cDNA by AMV First Strand cDNA Synthesis Kit (Bio Basic Inc. Canada), and the cDNA was amplified by PCR reagent (Taq polymase: MBI, USA; dNTP Mix: MBI, USA). Each 25 μl RT reactions contained 11.4 μl sterile and de-ionized H_2O; 2.5 μl of 10×PCR Buffer; 2.5 μl of dNTP mix (2 mmol/L); 1.25 μl each of forward and reverse IGF-I and GAPDH primers (10 μmol/L); 0.1 μl of TaqDNA polymase (5 U/μl); 1.5 μl of $MgCl_2$ (25 mmol/L); 2 μl of 125 pg-12.5 μg cDNA. The following procedure was used for amplication: 1 cycle of 95°C for 2 min; 30 cycles of 95°C for 45 s, 59.6°C for 1 min and 30 s, 72°C for 45 sec; and a final elongation step of 72°C for 10 min.

Ten μl of PCR products and 2 μl of loading dye (25% bromophenol blue, 25% glycerol) were mixed. Subsequently, PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide (0.5 μg/ml) for 1 h at 100 V. Low DNA mass ladder (MBI, USA) was used as a molecular weight marker. DNA bands were visualized and densitometric analysis was done on a UV transilluminator (UVP Bio-imaging System, USA).

The specificity of PCR primers for IGF-I and GAPDH genes was verified by examining the PCR amplicons using DNA sequence analysis (Shanghai Sangon Biological Engineering Technology & Service Co Ltd., China).

Statistical analysis

Values of maize stover and concentrate intake, plasma IGF-I, GH and insulin concentrations, and liver IGF-I mRNA level were analyzed using the General Linear Model procedure of SAS (SAS, 1985). The P-values less than 0.05 were used to determine significant differences between means in Duncan's multiple range tests.

RESULTS

Intra-duodenal infusion amounts of Met, Lys and Leu

Table 2 summarizes the data on the flows of nine essential amino acids through the duodenum and ileum, as well as their apparent digestibilities in the small intestine of growing goats (Experiment 1). Based on these data, it was calculated according to the formula of Shan and Tan (2004) that the infusive amounts of Met, Lys and Leu to balance AA profile in the small intestine were 0.77 g/d, 0.91 g/d and 0.58 g/d respectively. These data were used to formulate AA mixture in Experiment 2.

Dry matter intake of maize stover and concentrate

Intakes of maize stover and concentrate were unaffected ($p>0.05$) by the 21% replacement of Met, Lys or Leu with glutamate in Experiment 2. There was no significant difference ($p>0.05$) in total DM intake between four treatment groups (Table 3).

Plasma IGF-I, GH, insulin concentrations and liver IGF-I mRNA level

Effects of intra-duodenal supplementation of Met, Lys and Leu on plasma IGF-I, GH, insulin and liver IGF-I mRNA in growing goat are presented in Table 4 and Figure

Table 4. Effects of amino acids mixture infusion (control) and 21% replacement of individual methionine (-Met), lysine (-Lys) or leucine (-Leu) with glutamate in the duodenum on plasma IGF-I, GH, insulin and IGF-I mRNA/GAPDH mRNA in growing goats fed a maize stover-based diet

	-Met	-Lys	-Leu	Control	SEM
IGF-I (ng/ml)	13.8^{c}	14.2^{bc}	14.4^{bc}	16.5^{a}	0.51
Insulin (μIU/ml)	7.7^{b}	7.7^{b}	8.6^{ab}	9.3^{a}	0.40
GH (ng/ml)	0.85^{b}	0.89^{b}	0.98^{ab}	1.09^{a}	0.05
IGF-I mRNA /GAPDH mRNA	0.89^{c}	0.97^{bc}	1.15^{ab}	1.32^{a}	0.07

$^{a, b, c}$ See footnote for Table 3.

1. The plasma IGF-I concentration was reduced by the 21% replacement of Met ($p<0.01$), Lys ($p<0.05$), or Leu ($p<0.05$) with glutamate compared with the control group. The plasma GH and insulin concentrations were reduced by the 21% replacement of Met or Lys with glutamate, but the replacement of the 21% Leu with glutamate did not affect plasma GH and insulin concentrations compared with the control group ($p>0.05$). The IGF-I mRNA level in the liver was significantly reduced by the 21% replacement of Met ($p<0.01$) or Lys ($p<0.05$) with glutamate, but not by the replacement of Leu ($p>0.05$).

DISCUSSION

Maize stover, concentrate and total DM intakes

Results of this study showed that the replacement of the 21% Met, Lys or Leu with glutamate did not affect the intakes of maize stover, concentrate or total DM. The results were consistent with the findings of Robinson et al. (2000) who found that the intakes of organic matter (OM), DM and NDF were not altered by the abomasal infusion of Leu, Met or both in lactating cows. It is therefore, likely that the differences in plasma IGF-I, GH, insulin and liver IGF-I mRNA were mainly due to the amounts of the three limiting amino acids infused into the lumen of the duodenum.

Plasma IGF-I concentration

The study showed that the replacement of the 21% Met, Lys or Leu with glutamate markedly decreased plasma IGF-I concentration when compared with the control group in growing wether goats. This finding is in agreement with the results reported by other researchers. For example, Yang et al. (2000) reported high Lys intake increased serum IGF-I concentration of sow. Mejia-Guadarrama et al. (2002) noted that Lys restriction during lactation markedly reduced plasma IGF-I concentration of sows. Katsumata et al. (2002) found that plasma IGF-I level of porcine fed a low Lys diet was 52% lower than those of fed the control diet. Takenaka et al. (2000) reported Met, Lys and Leu deficiency reduced serum IGF-I content in rat. Katsumata et al. (2002) suggested that the potential mechanisms underlying the reduction in plasma IGF-I by imbalance of limiting amino acids include suppression of post-transcriptional events in IGF-I expression and differences in

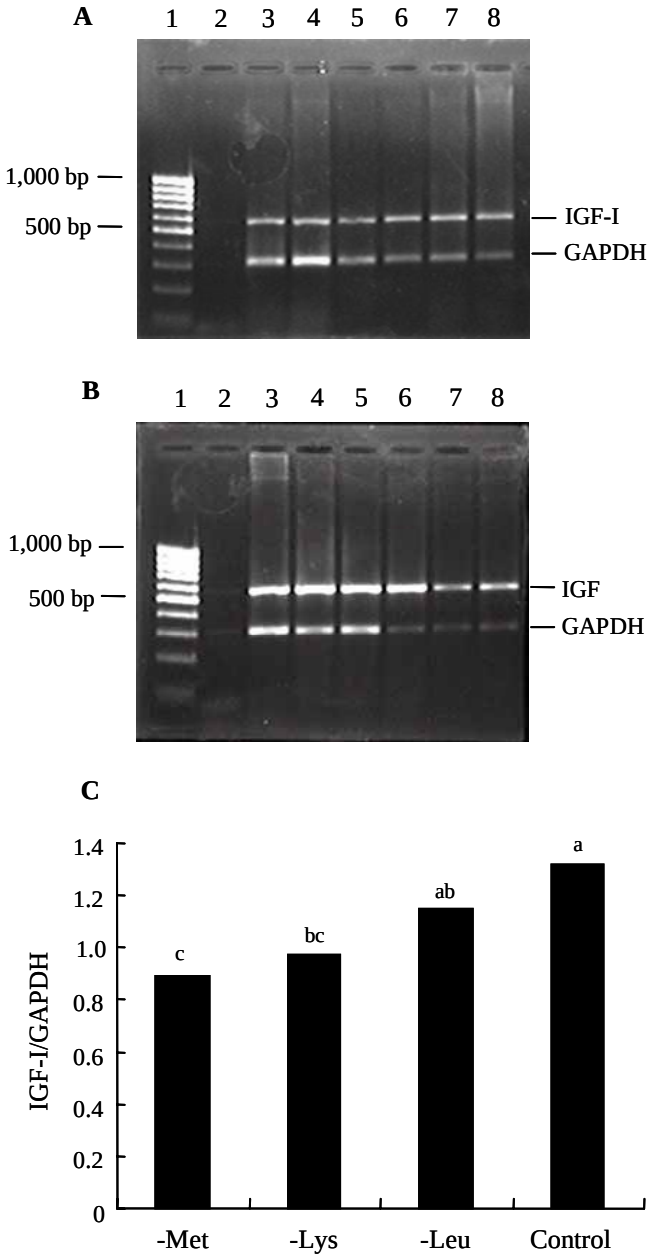

Figure 1. Effects of amino acids mixture infusion (control) and 21% replacement of individual methionine (-Met), lysine (-Lys) or leucine (-Leu) with glutamate in the duodenum on liver IGF-I mRNA level in growing goats fed a maize stover-based diet. Agarose gel electrophoresis (1.5%) shows RT-PCR products of IGF-I and GAPDH genes from liver. Lanes 1-8 represent separately DNA marker (1), negative control (2), replacement of Met (3-5) or Lys (6-8) in the picture A. In picture B, lanes 1-8 represent separately DNA marker (1), negative control (2), replacement of Leu (3-5) and control group (6-8). C: relative level of IGF-I mRNA, RT-PCR products of IGF-I from liver in 4 groups were normalized with the internal standard (GAPDH).

IGF-I clearance from plasma other than suppression of IGF-I mRNA expression. Injections of pharmacologic doses of GH to intact rats restricted in dietary protein for 1 wk restored hepatic IGF-I mRNA without normalization of serum IGF-I concentration (Thissen et al., 1991). This suggested that the reasons of lower plasma IGF-I in this study might involve suppression in transcription of the IGF-I. Although no direct evidence supporting a reduction in translation rate is available, Western ligand blotting showed that the principal binding protein of IGF-I in blood, $IGFBP_3$, was significantly reduced in pigs fed the lower Lys diet (Katsumata et al., 2002). The IGF-I-IGFBP3-acid labile subunit complex functions as a storage pool for circulating IGF and prolongs the half-life of IGF-I from <10 min in the free, unbound state to 15 h for the complex (Guler et al., 1989). These indicated increase of IGF-I clearance from plasma might the one of reasons for the decrease of plasma IGF-I influenced by imbalance of limiting amino acids at the duodenum. Insulin-like growth factor I is proposed to mediate the actions of GH on tissues (Gluckman et al., 1987), and measures of circulating concentrations of IGF-I have been proved to be positively correlated with growth in young ruminants (Olsen et al., 1981). Thus, the decrease in plasma IGF-I level in this study probably implies the reduction of growth in growing goats.

Plasma GH concentration

In this study, the replacement of 21% Met or Lys with glutamate reduced plasma GH concentration when compared with control group in growing wether goats. That amino acids could affect the plasma or serum GH has been shown previously. Bolze et al. (1985) reported serum GH in weaning rats was significantly decreased in Lys and Met deficiency groups. Supplementation of Lys and Arg, when infused intravenously or administered orally, could stimulate GH release in athletes (Chromiak and Antonio, 2002). Mejia-Guadarrama et al. (2002) reported Lys restriction during lactation markedly decreased plasma GH concentrations in sow. Compared with the replacement of Met or Lys, the replacement of 21% Leu with glutamate in the duodenum did not influence plasma GH concentration. GH affects almost all of the tissues and cells, such as immune tissue, brain tissue, and hematopoietic (Jing et al., 2001). The main actions of GH are the stimulation of growth and differentiation of bone and cartilage cell and the regulation of metabolism of protein, sugar and lipid (Denis et al., 1994). Thereby, in order to improve the growth performance, it is necessary to supply balanced duodenal limiting amino acids to maintain the higher plasma GH concentration in growing goats.

Plasma insulin concentration

This study proved that the replacement of 21% Met or Lys with glutamate in the duodenum decreased plasma insulin concentration, whereas the replacement of Leu had no significant effect in growing wether goats. The results were consistent with the previous repoerts. Yang et al. (2000) reported low Lys intake increased muscle protein degradation and decreased concentrations of serum insulin in sows. Mejia-Guadarrama et al. (2002) found Lys restriction during lactation markedly decreased plasma concentrations of insulin in sow. Rayland-Gray et al. (1997) reported increases of plasma insulin concentrations by supplementing Met in steers. Van de Ligt et al. (2002) noted insulin levels in growing pigs increased with the increasing levels of dietary Lys. Tokach et al. (1992) and Kusina et al. (1999) also observed reduction in the protein (Lys) content in the lactation diet resulted in a decrease in plasma insulin of sows. There was no difference in insulin concentration in venous blood of goats after hourly infusion of 0.72 mg of Leu (Puchala et al., 1995). From above, it can be concluded that the difference between groups in plasma insulin, is attributable to the infusion difference of amino acids (Met or Lys), since absorption of AA directly stimulates the pancreas to release insulin (Murray et al., 1999).

Insulin-like growth factor I mRNA level of the liver tissue

In this study, the replacement of 21% Met or Lys with glutamate in the duodenum reduced the level of liver IGF-I mRNA when compared with control group in growing wether goats. The results are in agreement with the reports followed. Harp et al. (1991) showed that IGF-I mRNA of cultured rat hepatocytes was decreased with the removal of Lys from the medium. Met was the key limiting amino acid involved in the modulation of IGF-I expression in the ovine liver (Stubbs et al., 2002). Compared with the effects of Met and Lys, a replacement of 21% Leu with glutamate didn't affect the level of liver IGF-I mRNA. So far, there has been no relevant report about the effects of Leu deficiency on the level of liver IGF-I mRNA.

Presently people are greatly interested in the mechanism of amino acids on liver IGF-I mRNA level. The point of view that restriction of limiting amino acids affects the quantity or binding activity of a number of liver-enriched transcription factors has been accepted generally (Marten et al., 1996; Nolten et al., 1996; Oka et al., 1997). According to the results from this study, it could be speculated that the decrease of liver IGF-I mRNA by reduced supply of Met or Lys was closely related to the reduction of plasma GH because GH has a regulatory function on IGF-I secretion (Daughad and Rotwein, 1989).

Possible nutritional-physiological mechanisms

The results that the amount of digested DM was not

significantly affected by a part-replacement of the limiting amino acids in this study, suggested that the energy supply may be similar between the groups. Therefore, possible effects of the level of energy intake on the hormone's regulation are not discussed in this paper.

Shan and Tan (2004) found when Met, Lys and Leu in the amino acids mixture infused at duodenum was respectively replaced with glutamate for the same N content basis, the reduction in N balance was the greatest for Met, followed by Lys and Leu when compared with the control, where all the three amino acids were infused to allow for amino acid profile of the digesta in the duodenum to meet the muscle amino acid profile. The results indicate that N deposition can be decreased by the inadequacy of these amino acids, further, protein anabolism, protein synthesis and degradation may be altered.

In this study, the effects of the limiting amino acids on the metabolic hormones (GH, IGF-I and insulin) could be resulted from their effects on production of these protein-hormones as for protein metabolism in general. Production, then secretion of these hormones could be changed, followed by their circulating concentrations which were induced by an inadequacy of amino acids supply. For example, circulating IGF-I is mainly produced in the liver, and the concentration increases with raised feed intake or nutrient supply (Clemons and Underwood, 1991; Underwood, 1996). The reduced supply of Met, Lys or Leu into the duodenum, then to the liver and the rest of the body could result in a decrease in amino acid substrate supply for synthesis of the hormone (Wheelhouse et al., 1999). For IGF-I, the amino acids could also selectively affect the response of its gene expression to GH. This has been found by Stubb et al. (2002) where the release of IGF-I in response to GH in cultured ovine hepatocytes was substantially higher in the media with amino acid concentrations 5 folds higher than normal physiological concentrations. In addition, IGF-I gene expression in the hepatocytes was almost abolished by reducing Met concentration in the media to 0.2 fold normal physiological concentration, lowered by the reduction of Lys, but slightly increased by the reduction of Leu (Stubb et al., 2002). The results in this study confirmed the lack of response of IGF-I mRNA in the liver of goats to a reduced supply of Leu. As IGF-I and insulin has an 'anti-catabolic'effect (as reviewed by Lobley, 1998), a decline of IGF-I and insulin concentrations would be associated with an increase in protein degradation which can release more amino acids from the peripheral tissues for important organs. This may compromise detrimental effects of reduced supply of the limiting amino acids on protein synthesis in these organs. Perhaps, this is a mechanism for animals to protect themselves from poor nutrition.

CONCLUSION

The results that the replacement of 21% limiting amino acids (Met or Lys) with glutamine, on a isonitrogenous basis, in the duodenum of growing goats significantly reduced plasma GH, insulin and IGF-I concentrations and liver IGF-I mRNA level, indicated that the influence of the limiting amino acids on nutrition of animals is likely intermediated via these hormones. The replacement of 21% Leu with glutamate reduced plasma IGF-I concentration, but neither not plasma insulin and GH, nor liver IGF-I mRNA level. The close relationships between supply of the limiting amino acids in the lumen of the duodenum and plasma IGF-I, GH and insulin concentrations, as well as liver IGF-I mRNA level indicate that these hormones could be used as intermediate markers for the limiting order of amino acids. Furthermore, appropriate dietary intake of limiting amino acids in growing goats is extremely important for the maintenance of normal levels of plasma IGF-I GH, insulin, liver IGF-I mRNA, which control optimal growth.

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