A Study on the Optimal Amino Acid Pattern at the Proximal Duodenum in Growing Sheep

Hongrong Wang* and Dexun Lu

Institute of Animal Nutrition and Feeds Science, Inner Mongolian Academy of Animal Science

Huhhot, Inner Mongolia 010030, P. R. China

ABSTRACT : Nine crossbred castrated lambs fitted with rumen and duodenum cannula and fed a diet of hay and concentrate formulated with ground corn and soybean meal as main ingredients were used to assess the duodenal ideal amino acid pattern. Three synthetic amino acid mixtures with different profile of essential amino acids were duodenally infused in order to get three different amino acid patterns flowing into the duodenum. The mixtures were designed to have similar amino acid profile as rumen microbial protein (Pm), casein (Pc) and modified muscle amino acid (Pmm). Results showed a lower urine nitrogen excretion ($p=0.05$), a higher nitrogen retention ($p=0.04$) and bodyweight gain with treatment Pmm. The modified muscle amino acid pattern also promoted a lower ratio of Gly to other amino acids in plasma (Gly/OAA) and a higher RNA and RNA/DNA concentration in the liver of the sheep. Meanwhile, the urea concentration in plasma was reduced and the insulin concentration was increased with Pmm treatment. No differences in glucose and growth hormone concentration in plasma were found among three treatments. All results obtained indicate that the modified muscle amino acid pattern (Lys 100%, Met+Cys 39%, Thr 76%, His 41%, Arg 72%, Leu 158%, Ile 81%, Val 105%, Phe 81% and Trp 13%) was the best for growing sheep. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 38-44)***

Key Words : Amino Acids, Duodenum, Growing Sheep

INTRODUCTION

The amino acid profile of the digesta flowing to the duodenum is closely related to animal production. It is now commonly accepted that ruminants, as well as non-ruminants, should be supplied at tissue level with sufficient essential amino acids. Therefore amino acid requirements and their balance presented for absorption from the gastrointestinal tract become an important consideration in ruminants just as nonruminants. The concept of "ideal protein" or "ideal amino acid balance" has been widely used in monogastric animals (Cole, 1979; Fuller et al., 1979; ARC, 1981; NRC, 1988). So far, the ideal amino acid pattern for ruminants has hardly been investigated and remains to be determined. In the case of the growing animals, a logical alternative would be to consider the amino acid pattern in carcass protein as the ideal protein (Meister, 1965), as was found to be the case with chicken and rats (Williams et al., 1954).

The objective of this experiment was to investigate the effect of different amino acid pattern at the proximal duodenum on nitrogen balance, plasma parameter and RNA/DNA concentration in the liver and longissimus muscle, and to investigate optimal amino acid pattern flowing into the duodenum of growing sheep.

MATERIALS AND METHODS

Animals, diet and feeding

Nine crossbred castrated lambs (Chinese Merino and Caucasian Merino, weighing between 25 and 30 kg), fitted with permanent rumen cannula and a T-shaped cannula at the proximal duodenum, were used in a completely randomized design with three treatment groups of three sheep each. Three synthetic amino acids mixtures with different profile of essential amino acids (table 1) were intraduodenally infused at rate of 6.443 g/d in order to get three different patterns of amino acids in the digesta flowing into the duodenum.

A: Rumen microbial protein amino acid pattern (Pm), as the ruminal microbial protein amino acid profile reported by McCance and Widdowson (1978).

B: Casein amino acid pattern (Pc), as the pattern of casein amino acid reported by Fraser et al. (1991).

C: Modified muscle protein amino acid pattern (Pmm), increased 50% of methionine and 50% of threonie) based on the muscle protein amino acid pattern in lamb (McCance and Widdowson, 1978).

Infused amino acids were calculated from an incremental model as follows:

$$Di(Qi+\Delta Xi)/(Dt\times Qt + \sum_{i=1}^{n} Di\times\Delta Xi\ ) = Ri$$

Where Ri refers to the amino acid profile in rumen microbial protein, casein and muscle protein. Di refers to digestibility of the i^{th} amino acid in the small intestine of the sheep; Dt refers to digestibility of total amino acid in the small intestine of the sheep. Qt refers to total amino acid flows to the duodenum of sheep; Qi refers to i^{th} amino acid flow to the duodenum of sheep. ΔXi is the infused amount of i^{th} amino acid. Dt and Di were determined using disappearance rate of amino acids flow between the

* Corresponding Author: Wang Hongrong. Tel: +86(0)-471-3960125, E-mail: nmxky@email.nm.cninfo.net

Received June 23, 2001; Accepted August 24, 2001

Table 1. Composition of duodenally infused amino acid mixture (g/d)

Amino acids	Pm	Pc	Pmm
L-Lys.HCl	1.711	0.736	2.037
DL-Met	0.372	0.636	1.180
L-Thr	1.058	0	0.441
L-His	0	0.333	0.016
L-Arg	0.594	0	1.882
L-Leu	0	0	0
L-Ile	0.165	0.434	0
L-Val	0	0.395	0
L-Phe	0.429	0.259	0
L-Trp	0.417	0.010	0.287
Na-Glu	1.696	3.641	0
Total N	6.443	6.443	6.443

* Amino acids were obtained from the Sigma Ltd.

duodenum and the ileum of sheep in companion study (Wang et al., 1998).

Treatment

The animals were housed individually in metabolic cages and given a mixed diet consisted of 62.5% chopped hay and 37.5% concentrate. The concentrate was formulated with 72% ground corn, 26% soybean meal (SBM), 1% bone meal, 0.5% salt and 0.5% of a mineral and vitamin premix. Concentrate (300 g/day per sheep, 18.2% CP/DM) and hay (500 g/day per sheep, 6.7% CP/DM) were fed in two equal portions at 06:00 and 18:00.

Experimental and sampling procedures

Chromic oxide (4 g/d Cr_2O_3 per sheep) was packed with filter paper and dosed 4 times per day during day 1 to day 14 via ruminal cannula as a nonabsorbable marker for measuring digesta flow.

The experimental period lasted 24 days with the first 8 days serving as an adjustment period, followed by 6 days for feed and feces sampling and duodenal digesta collection. For the digesta flow measurement, samples of duodenal digesta were collected 4 times per day at 6 h intervals on 6 consecutive days (day 8 through 14) to allow for a shift in sampling times. Digesta samples (30 ml) from the duodenum were obtained by removing the cannula plug and waiting for surges of digesta that was collected in whirl pack bags. The 24 individual samples of duodenal digesta were frozen, thawed later, pooled for each animal and lyophilized for later analysis.

From days 15 to 24, the experimental amino acids mixtures (dissolved in 720 ml sterile physiological saline and adjusted to pH 6.5) were infused continuously at a constant rate (0.60 ml/min) for 20 h each day. A medical infusion device was used to complete the duodenal infusion. At 8:00 a.m. (2 h after feeding) of the days 4, 7, 10 of the infusion period, jugular blood samples were collected in heparinized tubes by jugular puncture. A portion of plasma was deproteinized with an equal volume of 10% sulfosalicylic acid, the supernatant derived from centrifuging the mix was stored at -20°C used for determining plasma glucose, insulin, urea nitrogen (PUN), growth hormone and free amino acid (FAA) contents. Duodenal samples were collected three times on the days 3 to 5 of the infusion period. During day 5 to day 10 of infusion period, a nitrogen balance trial was performed. Total collection of feces and urine were performed twice every 24 h for 5 days. The output of feces and urine of the sheep was recorded, and aliquots were pooled for each sheep within period [5% (wt/wt) of daily feces and 10% (wt/wt) of daily urine]. Daily urine was collected in a bottle containing 200 ml of 20% (vol/vol) H_2SO_4 solution, and pooled urine was stored at 4°C until analysis, and pooled feces were dried at 65°C after spraying with 10 ml of 1 N HCl solution to retain volatile N components. At the end of the experimental period, all animals of each group were weighed and killed. Samples of *longissimus dorsi* muscle and liver were taken and stored at -60°C for later analysis of DM, nitrogen RNA, and DNA.

Analytical procedures

The N contents in feed, duodenal digesta and feces and urine were analyzed in duplicate with Kjedahl method (AOAC, 1984). The contents of moisture, ether extract and crude ash in feed, duodenal digesta and feces were also determined according to AOAC (1984). Chromium (Cr) content of duodenal digesta was measured as described by Gao Ming et al. (1993). Flows of DM (g/d) at the duodenum were calculated by dividing the amount of Cr dosed daily by the concentration of Cr in duodenal samples.

For amino acids analysis, samples (Ca 150 mg) of duodenal digesta were hydrolyzed in 6N·HCl for 22 h at 110°C. Amino acids were determined with a Hitachi 835 amino acid analyzer using ninhydrin as a coloring reagent. Test tubes were purged with nitrogen gas to minimize oxidation of sulfur amino acids during hydrolysis.

Growth hormone and insulin in plasma were determined by the radioimmuno-assay technique (RIA) (Kits were obtained from the North Biotech institute, Beijing, China). For tissue nucleic acid analysis, a homogenate (100 g/L) was prepared in 0.01 M phosphate buffer containing 0.15 M NaCl, 0.001 M EDTA, and sodium aside (0.2 g/L). A portion of the homogenate was transferred to a siliconized glass tube, perchloric acid (PCA) was added to a final concentration of 0.4 M. Sample tubes were placed in ice for 15 min before centrifugation at 1,000 g for 10 min at 4°C. The pellet was washed twice with 2 ml cold 0.4 M PCA. The final pellet was resuspended in 0.4 M PCA and

hydrolyzed for 20 min at 100°C and span again. The supernatant fraction was used for analysis of DNA and RNA concentrations. RNA contents in tissues were measured as described by Monro and Fleck (1969), and DNA contents in tissues were determined using the Hoerscht dye 33258 (Sigma product) (LaBarca and Paigen, 1980). Plasma urea nitrogen was tested by spectrophotometer (Ni Zhanming et al., 1990).

Statistical analyses

Body weight gain was calculated from the difference between initial and final weight of the experimental period. Data were analyzed according to the procedure of SAS (1987). If the interaction term was significant, differences among means were tested for significance with Duncan's multiple range tests.

RESULTS

Intake and digesta nutrient flows at the duodenum of sheep fed SBM diet before amino acids infusion are presented in table 2. No significant differences were found for amino acids and other nutrient flowing into the duodenum of sheep before amino acid infusion ($p>0.05$). Effects of duodenal amino acids pattern on the nitrogen balance and daily bodyweight gain during the infusion period are shown in table 3. Urinary nitrogen excretion was significantly lower ($p=0.05$) with Pmm than Pm, whereas nitrogen retention was significantly higher with Pmm ($p=0.01$) when expressed by metabolic body weight. The quotient retained N /digested N was also higher ($p=0.039$) with Pmm than Pm. The casein pattern (Pc) treatment showed intermediate values. Bodyweight gain was also affected ($p=0.05$) by the amino acid pattern in the duodenum of sheep. The highest bodyweight gain was observed in sheep on Pmm treatment (153.3 g/d). Nitrogen retention and bodyweight gain both shown lowest values in Pm treatment. As shown in table 4, total essential amino acids (EAA) concentration in plasma tended to be higher for Pmm treatment ($p=0.23$) than for Pm and casein treatments. Histidine and methionine in plasma were lower for Pm treatment compared with Pmm ($p=0.05$ and $p=0.14$, respectively). There were also significant differences in Gly to other amino acids ratio (Gly/OAA) with Pmm treatment showing a lower ratio than Pm treatment, which would indicate a better amino acid utilization with Pmm treatment. For other plasma amino acids, no clear pattern emerged as to how the response varied with amino acid pattern. The effect of duodenal amino acid pattern on RNA and DNA concentration in the liver and longissimus dorsi of sheep is shown in table 5. Amino acid pattern at the duodenum had no significant effect on the ratios of protein/RNA and RNA/DNA in the liver of sheep ($p=0.63$ and $p=0.72$, respectively), whereby with Pmm showing the higher values and Pm the lowest, whereas DNA concentration tended to be lower for Pmm. The responses of DNA or RNA observed in liver were much greater than that found in muscle. Mean concentration of plasma urea nitrogen (PUN), glucose, insulin and GH is shown in table 6. Plasma urea nitrogen was affected by different treatments, and was significantly lower with treatment Pmm ($p=0.05$). Glucose in plasma for Pmm treatment was fairly higher than that for Pm as well as casein treatments although no significant difference was found among three treatments ($p=0.06$).

Table 2. Nutrients flow at the duodenum of sheep before infusion of amino acids

	Pm	Pc	Pmm	SEM	P
DM intake (g/d)	670	721	711	27.02	0.81
N intake (g/d)	15.60	16.50	16.30	0.47	0.40
Total N flow at the duodenum (g/d)	16.10	17.70	17.90	0.99	0.36
DM flow at the duodenum (d/d)	503	586	564	43.20	0.21
AAs flow at the duodenum (g/d)					
Lys	5.18	6.47	5.93	0.65	0.61
Met	1.53	1.48	1.82	0.18	0.35
Thr	4.30	4.96	4.80	0.34	0.09
His	1.76	2.41	2.20	0.33	0.11
Arg	3.63	3.94	4.42	0.40	0.23
Leu	7.85	9.53	9.75	1.04	0.76
Ile	4.81	5.93	5.83	0.57	0.42
Val	5.67	6.41	6.79	0.57	0.34
Phe	4.46	5.47	5.40	0.56	0.28
Trp	0.63	0.85	0.69	0.11	0.46
EAA	39.83	47.46	47.63	4.46	0.78
NEAA	40.52	47.22	48.78	4.39	0.86

SEM=Standard error of mean.

Table 3. Effect of the amino acid pattern in the duodenal digesta on nitrogen balance and bodyweight gain of sheep

	Pm	Pc	Pmm	SEM	P
DMI (g/d)	517	598	720	12.65	0.86
Nitrogen intakes (g/d)	16.99^{b}	17.88^{ab}	19.30^{a}	0.71	0.09
Fecal nitrogen (g/d)	5.40	5.25	5.47	0.64	0.85
Urinary nitrogen (g/d)	7.16^{a}	6.35^{ab}	5.43^{b}	0.45	0.05
Digested nitrogen (DN) (g/d)	11.59	12.63	13.83	0.80	0.16
Retained nitrogen (RN) (g/d)	4.43^{b}	6.28^{ab}	8.41^{a}	0.78	0.04
Retained nitrogen (g/kgW$^{0.75}$)	0.34^{b}	0.48^{ab}	0.67^{a}	0.06	0.01
RN/DN (%)	38.24^{c}	49.47^{b}	60.51^{a}	2.03	0.039
Body weight gain (g/d)	60^{b}	146.7^{ab}	153.3^{a}	1.68	0.05

RN=retained nitrogen, DN=digested nitrogen. Means with different superscript differed significantly at $p<0.05$.
SEM=Standard error of mean.

Table 4. Effects of amino acid patterns in the duodenal digesta on the concentration of plasma free amino acid in sheep (μM)

AAs	Pm	Pc	Pmm	SEM	P
Lys	150.4	169.7	230.2	53.3	0.39
Met	42.3^{b}	59.8^{ab}	79.0^{a}	3.0	0.04
Thr	401.8	413.7	470.9	36.9	0.79
His	39.9^{b}	69.9^{ab}	76.6^{a}	8.2	0.05
Arg	146.0	163.5	255.9	64.3	0.56
Leu	123.1	158.2	189.2	41.2	0.38
Ile	88.3	118.9	131.5	27.4	0.40
Val	196.1	279.4	329.9	77.2	0.31
Phe	68.5	74.4	80.5	14.0	0.55
Asp	34.9	49.6	52.1	9.4	0.19
Ser	73.7	126.9	112.8	16.6	0.14
Glu	299.7	402.9	460.4	71.5	0.22
Gly	552.6	517.9	497.2	67.0	0.25
Ala	130.7	172.5	175.5	32.2	0.33
Tyr	56.5	59.9	63.5	22.4	0.93
Pro	81.2	74.6	115.4	18.9	0.39
EAA	1255.7	1429.7	1843.6	299.6	0.23
NEAA	1229.2	1319.8	1383.2	282.7	0.84
Gly/OAA (%)	28.61^{a}	22.93^{ab}	18.36^{b}	0.9	0.05

Means with different superscript differed significantly at $p<0.05$. SEM=Standard error of mean.

Plasma insulin concentration in Pmm treatment group was increased significantly ($p=0.04$) compared with Pm and casein treatments. Growth hormone levels among three treatments were not significantly different ($p=0.31$). Daily nitrogen retention (Y g/d) showed a high linear correlation ($r=0.808$) with plasma insulin concentration (X. mU/l).

DISCUSSION

The aim of this study was to determine the optimum pattern of amino acid flowing into the duodenum of growing sheep under the hay and mixed concentrate based diet. The amino acid pattern is highly relative to the utilization of absorbed essential amino acids in the ruminants. At present study, amino acid flow into the duodenum of sheep was estimated before the infusion of synthetic amino acids mixtures. The mathematical model was introduced to estimate how much individual amino acid needs to be infused. Apparent disappearance of amino acid from the intestine had been determined previously (Met 80.8%, Lys 77.0%, Thr 64.0%, Ile 73.8%, Leu 81.5%, Val 76.4%, Phe 78.8%, Arg 71.1%, His 74.0% and Trp 76.0%, respectively) (Wang, 1998), which was used in current experiment. Chromium oxide used to estimate the digesta flow in this experiment definitely has some drawback compared with double marker system, but indeed it is one of simple marker system that had been frequently used in many studies especially in large ruminant animals (Cecava et al., 1990; Klusmeyer et al., 1990; Ludden et al., 1995). Amino acid profile was designed to have different pattern in

Table 5. Effect of amino acid pattern in the duodenal digesta on RNA and DNA concentration in the liver and longissimus muscle of sheep

	Pm	Pc	Pmm	SEM	P
Liver					
Protein (%)	19.94	20.89	20.46	0.96	0.75
RNA (mg/g wet sample)	0.71	0.75	0.77	0.08	0.93
Protein/RNA (g/mg)	0.28	0.28	0.26	0.01	0.63
DNA (mg/g wet sample)	2.74	2.91	2.48	0.39	0.76
Protein/DNA (g/mg)	0.07	0.07	0.08	0.01	0.72
RNA/DNA	0.26[b]	0.26[b]	0.32[a]	0.09	0.24
Longissimus muscle					
Protein (%)	19.28	20.00	18.60	0.70	0.65
RNA (mg/g wet sample)	0.44	0.43	0.39	0.03	0.88
Protein/RNA (g/mg)	0.44	0.47	0.48	0.03	0.86
DNA (mg/g wet sample)	1.00	0.67	1.01	0.12	0.52
Protein/DNA (g/mg)	0.19	0.30	0.18	0.07	0.62
RNA/DNA	0.44	0.64	0.39	0.01	0.71

Means with different superscript differed significantly at $p<0.05$. SEM= Standard error of mean.

Table 6. Effect of the amino acid pattern in the duodenal digesta on PUN. glucose, insulin and growth hormone concentrations in plasma of sheep

	Pm	Pc	Pmm	SEM	P
PUN (mg/l)	162.8[ab]	186.2[a]	151.3[b]	1.2	0.05
Glucose (mg/l)	747.6	709.5	896.2	16.0	0.06
Insulin (mU/l)	4.70[c]	8.20[b]	14.23[a]	0.63	0.04
Growth hormone (µg/l)	1.43	0.97	1.43	0.29	0.31

Means with different superscript differed significantly at $p<0.05$. SEM=Standard error of mean.

current experiment based on the proposed model. whereas with the same level of total infused amino acids (6.44 g/d). These were attempting to make amino acid profile and total amino acid flow affect in different way to the studied parameters. The basal amino acid flows were not significantly different among sheep before synthetic amino acid infusion (table 2). The total amino acid flow was supposed to be stable during amino acid infusion; any amino acid profile changed should be derived from infused amino acids. The responses to nitrogen balance of the animals reflect the amino acid pattern presented during treatment period. The modified amino acid pattern was designed based on muscle protein amino acid pattern in lamb with exception of increase of 50% of methionine and 50% of threonine. as methionine and threonine are proved the first and second limiting amino acid by a companion study (Wang, 1999). The present study showed that the modified amino acid pattern (Pmm) resulted in higher retained nitrogen or RN/DN. which was mainly caused by decreased urinary nitrogen excretion, whereas less difference occurred in fecal nitrogen output. The nitrogen intake difference among three treatments mainly arose from the dry matter intake (DMI). the improved pattern of amino acids available for absorption in the gut of sheep would result in increasing roughage intake as reported by Leng (1982). Harper (1964) also reported that amino acid imbalance for absorption cause lower intake in monogastric animal. The higher nitrogen retention and bodyweight gain with Pmm treatment would indicated that it was the optimum one in three tested amino acid pattern and could be envisage as an ideal amino acid pattern for growing sheep. The body weight gain of the animals during the treatment period shown a higher value for Pmm treatment. although a short time response might not be a reliable index. Free amino acid concentration in plasma was frequently used to monitor the status of amino acid balance and utilization in animals (Hogan et al., 1968; Egan. 1972; Reis et al., 1973; Fenderson et al., 1975; Youny et al., 1981; D'Mello, 1994). In current study. methionine. histidine, serine and proline were given some obvious changes in response to three patterns of amino acid in the duodenum of sheep. Histidine and metionine in plasma were significantly lower for Pm treatment compared with Pmm ($p=0.05$ and $p=0.04$ respectively). which might reflect the shortage of methionine and histidine in the rumen microbial protein (Storm. 1982; Storm and Ørskov. 1984). Plasma glycine has been shown to decreased for Pmm treatment. which indicate nitrogen or amino acid status of the sheep in present trial were improved as Gly/OAA appears to be indicative of nitrogen status in animals (Egan. 1972; Reis et al., 1973).

The plasma glucose, insulin and growth hormone levels are related to growth rate and protein turnover and amino acid incorporation in muscle (Reeds and Davies, 1992). The enhancement of glucose and insulin obtained for Pmm in this trial was corresponded with the N balance results. It might be explained that Pmm treatment present a balanced amino acid pattern for absorption, which made plasma insulin level to increase and nitrogen retention rate improve subsequently. Plasma urea N levels are also an indication of the protein status of an animal (Pfander et al., 1975). The PUN result of Pmm is also a reflection of the balanced amino acid pattern. The reduced plasma urea N indicates that oxidation of amino acids leaving the pool indeed declined with Pmm treatment. The decreased PUN concentration ($p=0.05$) for Pmm treatment implies that balance of absorbed amino acid at the small intestine was helpful to improve the efficiency of amino acid nitrogen utilization in animal tissues, which made PUN concentration dropped accordingly. Measurement of tissue protein and nucleic acids was made to suggest cellular mechanisms responsible for the changes in response to amino acid patterns in the duodenum of sheep. RNA/DNA ratio and RNA concentration in the liver and muscle were used as an effective index in evaluating the potential capacity of protein synthesis of animals (Trenkle et al., 1978; Eversole et al., 1981; Reeds et al., 1986; Beermann et al., 1987). However, different amino acid pattern did not influence RNA/DNA ratio or RNA concentration in both the liver and muscle significantly in current experiment. Protein/RNA ratio implies translation efficiency in the process of protein synthesis, but protein/RNA in both the liver and muscle were not affected by amino acid pattern. Hence, we have no evidence supporting the statement in current experiment.

An optimal amino acid pattern

Microbial protein and casein patterns had been used as reference patterns (Fraser et al., 1991; Storm and Ørskov, 1984). Those studies were undertaken through the intragastric infusion method where sole nitrogen source was supplied by isolated rumen microbial protein or casein. However, the results obtained in current study are based on practical diet and measurement of digesta flow. The measured amino acid patterns of digesta at the proximal duodenum during duodenal amino acids infusion are shown in table 7. The present data are in form of digested amino acid pattern, which could not be simply compared to combination of table 1 and table 2 because infused amino acids did not involve any amino acid digestibility. Overall, the proposed optimal amino acid pattern in this study proved as a modified muscle amino acid pattern, which resulted in improving the retained nitrogen, bodyweight gain and blood index.

It is conclude that the modified pattern in this study can be regarded as the best amino acid pattern of growing sheep, which shows in percentage of Lysine as 100, Met+Cys 39, Thr 76, His 41, Arg 72, Leu 158, Ile 81, Val 105, Phe 81, Trp 13, EAA/NEAA 1:1.01. This pattern might be useful for manipulation of amino acid profile in the digesta flowing into the duodenum of sheep to enhance growth performance and reduce urinary nitrogen excretion. In addition, current results were obtained only under designed diet with soybean meal as main nitrogen source, and therefore the ideal amino acid pattern for growing sheep will need further examination in different diets.

ACKNOWLEDGMENTS

Our thanks to Mrs. Zhang Haiying, Mrs. Zhao Xouying, Mrs. Yijing and Miss Wu Rina for their assistance of sampling and analyses in Inner Mongolian Academy of Animal Science. We thank Dr Shimin Liu for useful discussions and advice of our experiment design. We are also grateful to the financial aid with the project was

Table 7. Measured digestible amino acid patterns in the digesta at the proximal duodenum during duodenal amino acid infusion (%)

AAs	Pm	Pc	Pmm	SEM	P
Lys	(6.791)100	(7.134)100	(6.706)100	0.05	0.35
Met	20	14	17	1.05	0.35
Thr	75[a]	67[b]	76[a]	0.86	0.05
His	41	42	41	2.61	0.85
Arg	65	61	72	0.68	0.15
Trp	11	12	13	1.15	0.56
Leu	157	149	158	10.15	0.58
Ile	85[a]	78[b]	81[ab]	0.55	0.08
Val	105	98	105	1.54	0.38
Phe	81	86	81	8.93	0.75
Cys	14	14	22	8.74	0.50
EAA/NEAA	1.00	1.01	1.00	0.03	0.88

Means with different superscript differed significantly at $p<0.05$. SEM=Standard error of mean.

provided by Inner Mongolian Committee of Science and Technology. This paper is from a part of the Ph.D. dissertation of Wang Hongrong (Inner Mongolia Agricultural University, P. R. China. 1998).

REFERENCES

AOAC, 1984. Official Methods of Analysis (13th Ed). Association of Official Analytical chemists, Washington DC.

ARC. 1981. The nutrient requirement of pig. Slough: Commonwealth Agricultural Bureau.

Beermann, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65:1514.

Cecava, M. J., N. R. Merchen, L. L. Berger and Jr. Fahey. 1990. Intestinal supply of amino acids in sheep fed alkaline hydrogen peroxide-treated wheat straw-based diets supplemented with soybean meal of combination of corn gluten meal and blood meal. J. Anim. Sci. 88:467.

Cole, D. J. A. 1979. Amino acids nutrition of the pig. In: Recent Advances in Animal Nutrition-1978. [W. Haresign and Lewis, editors], London: Butterworths pp. 59-72.

D'Mello, J. P. F. 1994. Amino acid imbalances, antagonisms and toxicities. In: Amino Acid in Farm Animal Nutrition (Ed. J. P. F. D'Mello).

Egan, A. R. 1972. Plasma and urinary metabolites as indices of nitrogen utilization in sheep. Proc. New Zealand Soc. Anim. Prod. 32:87.

Eversole, D. E., W. G. Bergen, R. A. Merkel, W. T. Magee and H. W. Harpster. 1981. Growth and muscle development of feedlot cattle of different genetic backgrounds. J. Anim. Sci. 53:91-101.

Fenderson, C. L. and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759.

Fraser, D. L., E. R. Ørskov, F. G. Whitelow and M. F. Franklin. 1991. Limiting amino acids in dairy cows given casein as the sole source of protein. Livest. Prod. Sci. 28:235-252.

Fuller, M. F., R. M. Livingstone, B. A. Baird and T. Atkinson. 1979. The optimal amino acid supplementation of barley for the growing pig. 1. Response of nitrogen metabolism to progressive supplementation. Br. J. Nutr. 41:321-331.

Gao Ming and Feng Zhongzi. 1993. A modified method for the analysis of chromium in feeds and faces samples. Inner Mongolian Anim. Sci. Prod. 3:42-43.

Harper, A. E. 1964. Amino acid toxicities and imbalance. In: Mammalian Protein Metabolism (Ed. H. N. Muaro and J. B. Allison). Vol. 1. Academic Press. New York.

Hogan, J. P., R. H. Weston and J. R. Lindsay. 1968. Influence of protein digestion on plasma amino acids level in sheep. Australian J. Biol. Sci. 21:1263.

Klusmeyer, T. H., Jr. R. D. Macarthy, J. H. Clark and D. R. Nelson. 1990. Effect of source and amount of protein on ruminant fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 73:3562.

LaBarca, C. and K. Paigen. 1978. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. 102:344.

Leng, R. A. 1982. Modification of rumen fermentation. In: Nutritional Limits to Animal Production from Pastures (Ed. J. B. Hacker). Commonw. Agric. Bur., Farnham Royal, U.K.

Ludden, N. A. and M. J. Cecava. 1995. Supplemental protein sources for steers fed corn-based diets: 1. Rumial characteristics and intestinal amino acid flows. J. Anim. Sci. 73:1324-1333.

McCance, R. A. and E. Widdowson. 1978. The Composition of Foods. 4th ed (Ed. D. A. T. Southgate and A. A. Paul). HMSO, London.

Meister, A. 1965. Biochemistry of Amino Acids. Vol. 1, Academic Press, New York.

Monro, H. N. and A. Flack. 1969. Analysis of nitrogenous constituents. Measurement of nucleic acids in tissues. In: Mammalian Protein Metabolism. Academic Press, NY. Vol. 3, p. 477.

Ni, Zhanming and Qiu Bili. 1990. A procedure of assessing plasma urea nitrogen in biological samples. In: Clinic Biochemical Test. Shanghai Science and Technology Press, Shanghai.

NRC. 1988. Nutrient requirements of swine. 9th. Rev. Ed Washington DC. National Academy Press.

Pfander, W. H., S. E. Grebing, C. M. Price, O. Lewis, J. M. Asplund and C. V. Ross. 1975. Use of plasma urea nitrogen to vary protein allowances of lambs. J. Anim. Sci. 41:647.

Reeds, P J., S. M. Hay, P. M. Dorwood and R. M. Palmer. 1986. Simulation of muscle growth by clenbuterol: lack of an effect on muscle protein biosynthesis. Br. J. Nutr. 56:248.

Reeds, P. J. and T. A. Davies. 1992. Hormonal regulation of muscle protein synthesis and degradation. In: The Control of Fat and Lean Deposition (Ed. P. J. Buttery, K. N. Boorman and D. B. Lindsay). pp. 1-26.

Reis, P. J., D. A. Tunks and L. F. Sharry. 1973. Plasma amino acid pattern in sheep receiving abomsal infusion of methionine and cystine. Australian J. Biol. Sci. 26:635.

SAS, 1987. SAS User's Guide, Statistics, Version 6 Edition, SAS Ins. Inst., Cart, NC.

Storm, E. 1982. Isolation and nutritive value of rumen microorganisms and their limiting amino acids for growing sheep. Ph.D. Thesis. University of Aberdeen. p. 486.

Storm, E. and E. R. Ørskov. 1984. The nutritive value of rumen microorganisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determining by a new approach. Br. J. Nutr. 52:613-620.

Trenkle, A. and D. G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. J. Anim. Sci. 46:1604-1609.

William, H. H., L. Curtin, J. Abraham, J. K. Loosli and L. A. Maynard. 1954. Estimation of growth requirements for amino acids by assay of the carcass. J. Biol. Chem. 208:277.

Wang, H. R, D. X. Lu, H. Y. Zhang and X. Y. Zhao. 1998. Determination of amino acid digestibility in the small intestine of growing sheep. Inner Mongolian Journal of Animal Science and Production. 19(2):18-21.

Wang, H. R. and D. X. Lu. 1999. A Study on Limiting Amino Acids for Growing Sheep Fed a Corn Based Diet. Journal of Animal Nutrition (ACTA ZOONUTIMENTA SINICA). 11(4):17-28.

Youny, A. W., G. T. Schelling, E. R. Tucker and Jr. G. E. Mitchel. 1981. Plasma amino acids response to abomasal infusion of amino acids in sheep. J. Anim. Sci. 52:1421-1429.