Effects of dietary ratio of ruminal degraded to undegraded protein and feed intake on intestinal flows of endogenous nitrogen and amino acids in goats

Chuanshe Zhou, Zhiliang Tan*

Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha 410125, China
Tel.: +86 731 84619702; Fax: +86 731 84612685; E-mail address: zltan@isa.ac.cn.

Abstract: Four goats (20 ± 2.5 kg) fitted with the ruminal, duodenal and ileal cannulaes were used in a 2 × 2 factorial design to estimate the effects of dietary ratio of ruminal degraded protein (RDP) to ruminal undegraded protein (RUP) and feed intake on the duodenal and ileal flows of endogenous nitrogen (EN) and endogenous amino acids (ENAA) in goats. Goats were fed two diets with dietary RDP to RUP ratios of 65:35 and 45:55 (RDP1 and RDP2, respectively), and fed at 95 and 75% of voluntary feed intake levels (DMI1 and DMI2, respectively). For the four treatments, the duodenal flows (g/d) of EN and ENAA were 1) 1.81, 4.16; 2) 1.67, 4.04; 3) 1.73, 3.87; 4) 1.10, 3.31, and the ileal flows (g/d) of EN and ENAA were 1) 0.20, 2.34; 2) 0.13, 1.92; 3) 0.15, 2.74; 4) 0.13, 2.24, respectively. The intestinal re-absorption (%) of EN and ENAA for the four treatments were 1) 77.4, 77.0; 2) 92.7, 77.2; 3) 86.5, 80.0; 4) 84.5, 77.0, respectively. The duodenal and ileal flows of EN and ENAA decreased by about 22 and 9%, 35 and 22%, respectively, when the feed intake changed from DMI1 (0.63 kg/d) to DMI2 (0.50 kg/d). The present results implied that the duodenal flows of EN and ENAA decreased when dietary RDP to RUP ratio and DMI decreased, and the flow of ENAA at the ileum also decreased when DMI decreased, whilst increased with decreasing RDP to RUP ratios.
[Chuanshe Zhou, Zhiliang Tan. **Effects of dietary ratio of ruminal degraded to undegraded protein and feed intake on intestinal flows of endogenous nitrogen and amino acids in goats.** *J Am Sci* 2014;10(4s):79-87]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 10

Key words: Ruminal degraded protein, Feed intake, Endogenous nitrogen, Goats

1. Introduction

The endogenous nitrogen (EN) can contribute significantly to the ruminal N pool which is necessary for the rumen microbial growth (Egan et al., 1986; van Bruchem et al., 1997). However, the EN contribution in the small intestine of ruminants is usually neglected and has not received much attention until recently. But there is a tendency towards acknowledging that the endogenous protein makes up a considerable fraction of duodenal N flow (Larsen et al., 2000; NRC, 2001). Determination of EN along the gastrointestinal tract allows for a better adjustment of both supply and requirement for N and amino acids (AA) in ruminants. The knowledge of EN loss permits a more accurate estimation of true N digestibility and is necessary to know how much dietary N is needed to cover the requirement for EN and endogenous AA (ENAA) loss (Hess et al., 2000; Sève and Hess, 2000; Ouellet et al., 2002).

Different methods such as the isotope dilution method (Simon et al., 1983; Ouellet et al., 2002), the difference method (Larsen et al., 2000), the amino acids profile (AAP) method (Powell, 1964; Evans et al., 1975; Larsen et al., 2000; Jensen et al., 2006) and the water soluble method (Larsen et al., 2001) have been used to determine losses of EN and ENAA along the gastrointestinal tract in ruminants. Ouellet et al. (2002) set up models to estimate EN losses for the pre-intestinal, intestinal and the total gastrointestinal tract using the ^{15}N isotope dilution method in dairy cows. Lapierre et al. (2008) established a model to estimate the intestinal ENAA losses in dairy cows. The difference and AAP methods were developed (Powell, 1964; Evans et al., 1975; Larsen et al., 2000) by separating the proportions of N and AA passing the duodenum in microbial, endogenous and undegraded feed protein. Larsen et al. (2000) developed the water soluble method which assumed the endogenous protein fraction at the ileum was located in the water soluble phase.

The loss of EN or ENAA is influenced by several factors such as animal species, feed intake (Tamminga et al., 1995; Ludden and Kerley, 1997; Nyachoti et al., 1997), dietary fiber content (Zebrowska and Kowalczyk, 1991; Tamminga et al., 1995), dietary protein level and its components (Bunting et al., 1989). However, the AA composition of the endogenous protein at the duodenum and ileum has received few available data in goats, and few available data has been reported about the effects of RDP to RUP ratio on flows of EN and ENAA in ruminants. Additionally, accurate estimates of EN and ENAA along the digestive tract are essential for the optimization of protein nutrition in ruminants. Therefore, the objective

of the present study was to estimate the effects of dietary ratio of RDP to RUP, and feed intake levels on flows of EN and ENAA at the duodenum and ileum in goats.

2. Materials and methods

2.1 Animals and management

The experiment was conducted according to the animal care guidelines of the Animal Care Committee, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha city, Hunan province, China.

Four Liuyang black wethers (a local breed in the South of China) with an initial body weight 20 ± 2.5 kg were fitted with ruminal plastic cannula (4-cm i.d.) and proximal duodenal and terminal ileal fistulae (T-type, 1-cm i.d., Ningxia University, Laboratory Factory, Yinchuan, China). The animals were kept individually in stainless metabolic cages in a temperature-controlled (21℃) and constant-lighted animal house, with free access to fresh water.

2.2 Experimental diets and design

Four goats were randomly assigned to one of four treatments in a 2 × 2 factorially arrangment with two dietary RDP to RUP ratios (65:35 and 45:55, namely RDP1 and RDP2, respectively) and two feed intake levels (95% and 75% of its voluntary feed intake, namely DMI1 and DMI2, respectively). The ingredients and chemical composition of the experimental total mixed rations (TMR) are presented in Table 1. Before the formal experiment commenced, all goats were fed on their respective experimental TMR diet ad libitum for two weeks, and daily intake and residue were recorded to measure the voluntary feed intake (VFI). The average VFI for the goats during this period was 0.66 kg/d (DM basis). This value was taken in 100% VFI and thereafter the goats were fed either 95% or 75% of this as equal portions at 07:00 and 19:00 h daily. The experimental period was lasted for 24 d, consisting of 14 d for adaption, 3 d for *in situ* degradability of dietary protein determination and 7 d for sample collection.

2.3 Sample collection

Feed samples were taken before feeding during the collection period. The procedure to measure ruminal degradability of dietary crude protein was according to the study of Tan et al. (2001) during d 15 to 17. From d 18 to 24, a total of 4 g chromic oxide (Cr_2O_3: 1 g every 6 h; particulate-phase digesta marker), was administered daily via the rumen fistulae at 06:00, 12:00, 18:00 and 24:00 h, respectively. From d 18 to 21, total feces and urine were collected according to the method reported by Zhao et al. (2009). In order to measure the digesta flows at the duodenum and ileum, 50 ml ruminal, 50 ml duodenal and 30 ml ileal samples were collected at 05:00, 11:00, 17:00 and 23:00 h on d 22, at 03:00, 09:00, 15:00 and 21:00 h on d 23 and at 01:00, 07:00, 13:00 and 19:00 h on d 24, respectively. At the end of the experiment, equal portions of the samples were pooled over the whole period for later analyses. The ileal samples were chilled to 5 ℃ until all 12 samples of each goat were collected to avoid hydrolysis of bacteria, which would give cell content to the water soluble phase.

2.4 Sample handling

One subsample of the pooled ruminal digesta samples was used to isolate bacteria by differential centrifugation according to the procedure of Reed et al. (2004) for analyses of DM, N diaminopimelic acid (DAPA) and AA. About 100 g of pooled duodenal and ileal pooled digesta were freeze-dried (GLZY-0.5B, Pudong Freeze Dryer Equipment Co., Ltd., Shanghai, China) and analyzed for DM, N, DAPA and AA. 100 ml demineralized water was added to another 100 g of pooled ileal digesta, then shaken for 5 min and suspension was strained through two layers of cheesecloth. The remaining feed particles and protozoa were removed by centrifugation (409 ×g, 5 min, 3℃). The bacterial fraction in the ileal digesta was removed by centrifugation of the supernatant twice (17,300 ×g, 20 min, 3 ℃), and the supernatant was frozen for the analysis of water soluble N and AA (Larsen et al. 2001). A 50 g representative sample of daily feces was frozen at -20℃ for later analyses. Urine was acidified daily with 50 ml 1.5N H_2SO_4. Subsamples of urine were taken each day and kept at -20℃ until analysis.

2.5 Chemical analyses

The collected samples were oven dried (65℃), air equilibrated, and ground to pass through a 1-mm sieve (DF-2, Changsha Instrument Factory, Changsha, Hunan, China). DM contents of feed, orts, feces, digesta and nylon bag residues were determined by drying at 65℃ for 48 h. Total N contents of feed, feces, urine, digesta and incubated nylon bag samples were analyzed according to the methods of AOAC (2002). The bacterial N was determined by the Dumas method described by Hansen (1989). The AA contents including DAPA were determined by the procedure of Mason et al. (1980). The concentration of chromic oxide on digesta was determined colorimetrically after oxidation with chromate according to Schurch et al. (1950).

2.6 Calculations

Effective rumen degradability (ERD) and fractional degradation rates of N or AA were calculated using a nonlinear model as described by Øskov and McDonald (1979):

$$Y = a + b \times (1 - e^{-ct}) \quad (1)$$

$$ERD = a + bc/ (c + K_p) \quad (2)$$

Where Y = the potential disappearance at time t, a = the rapidly soluble fraction washing out at 0 h, b = the potentially degradable fraction, c = the constant rate

of degradation of fraction b, t = the time. The passage rate (Kp) to calculate the ERD was estimated according to the following equation described by NRC (2001): Kp (%/h) = 3.362 + 0.479 × (DMI, % of BW) – 0.017 × (% NDF, DM basis) – 0.007 × (% concentrate in diet, DM basis) derived from each animal`s actual DMI and BW. The calculated Kp values were 0.051 and 0.040 %/h for DMI1 and DMI2 treatment in this study.

The content of RUP was calculated as follows:

RDP = ERD × DP (3)

RUP = 1- RDP (4)

Where DP was the content of dietary N or AA.

The flow of DM, N and AA at the duodenum and ileum was calculated as described by Sun et al. (2007). The duodenal flows of microbial N was determined by the internal microbial marker DAPA (Hutton et al., 1971), where the microbial passage at the duodenum was calculated from the concentration of DAPA in isolated rumen bacteria and the passage of DAPA at the duodenum. The following equation was used to calculate the duodenal flow of microbial N and AA:

$$R = \frac{DPd / Nd}{DPm / Nm} \quad (5)$$

Mp = R × DFN (6)

Where R means the ratio of microbial N to total N or AA of digesta, DPd and Nd stand for the concentration of DAPA and N in the digesta, respectively. DPm and Nm are the concentrations of DAPA and N in isolated rumen bacteria. Mp is the flow of microbial N at the duodenum. DFN stands for the flow of N in the digesta at the duodenum.

The duodenal flow of EN was calculated by the difference method (Larsen et al. 2000) according to the following equation:

Endogenous flow = total flow – microbial flow – undegraded feed flow (5)

Duodenal flow of individual AA was also separated by the amino acid profile (AAP) method (Larsen et al., 2000; Powell, 1964; Zhou et al., 2008). This mathematical method can estimate the contribution of total AA from each origin by solving the following equation using the least squares calculation of PROC REG (SAS, 1996):

$AA_i = \beta_1 \times FeedAA_i + \beta_2 \times MicAA_i + \beta_3 \times AboAA_i + \beta_4 \times BileAA_i$ (6)

Where AA_i is the *i*th amino acid flow in the duodenum; *i* is the individual amino acid (*i* = 1-16); β_{1-4} is total AA from each origin; $FeedAA_i$, $MicAA_i$, $AboAA_i$ and $BileAA_i$ are the *i*th AA proportion in undegraded protein in TMR, microbial and endogenous protein in abomasum and bile, respectively. The average contribution of endogenous AA secreted prior to the small intestine was estimated by the use of an AA profile of abomasal fluid and bile collected from slaughtered goats (Zhou et al., 2008).

The ileal EN and endogenous AA were assumed to be located in the water soluble phase and the apparent re-absorption of EN and endogenous AA was calculated according to the method of Larsen et al. (2001).

2.7 Statistical analysis

Data were analyzed using the General Linear Models procedure (SAS, 1996). Multiple comparisons of least-squares means were analyzed by Duncan's option. The model used was:

$Y_{ij} = \mu + RDP_i + DMI_j + (RDP \times DMI)_{ij} + \varepsilon_{ij}$ (7)

Where Y_{ij} was the response, μ was the overall mean, RDP_i was the mean effect of level *i* of dietary ratio of RDP to RUP (65:35 or 45:55), DMI_j was the mean effect of level *j* of feed intake (95 or 75% of the voluntary feed intake), $(RDP \times DMI)_{ij}$ was the associated interaction of dietary ratio of RDP to RUP and feed intake level effects, and ε_{ij} was the random residual error and assumed N (0, σ^2). Multiple comparison of least-squares means were analyzed by Tukey's option. Significant statistical effects were declared when probabilities (*P*) were below 0.05 and tendencies were considered in $0.05 < P < 0.10$.

3. Results

All the goats were healthy and consumed their feed allowance throughout the experiment.

The duodenal flow, ileal flow and intestinal re-absorption of EN are presented in Table 2. There were no significant differences ($P > 0.05$) for N intake, duodenal flow of total N (TN), undegraded feed N (UFN), microbial N (MN), EN, ileal flow or intestinal re-absorption of EN. The average ratio of EN to TN at the duodenum were 17.1% and 14.4%, when the goats were fed the RDP1 and RDP2 diets respectively. The average re-absorption of EN in the intestine was about 85.3%.

The effect of dietary ratio of RDP to RUP and feed intake on the flow of endogenous AA at the duodenum and ileum and intestinal re-absorption are shown in Table 3. The dietary ratio of RDP to RUP had significant effects ($P < 0.05$) on the ileal flows of endogenous leucine and cysteine. The feed intake had significant effects ($P < 0.05$) on the intestinal re-absorption of endogenous isoleucine, leucine, lysine and tyrosine. The dietary ratio of RDP to RUP and feed intake had no significant effects ($P > 0.05$) on the intestinal re-absorption of threonine, while there was significant interaction effects ($P < 0.05$) on the intestinal re-absorption of endogenous threonine. The ileal flow of endogenous phenylalanine was significantly affected ($P < 0.05$) by dietary ratio of RDP to RUP and feed intake, but no interaction effect ($P > 0.05$) was observed.

4. Discussion

4.1 The duodenal flow of EN and endogenous AA

It has been demonstrated that dietary protein is the factor determining the EN secretion in the gut (Schneeman, 1982; Darragh et al., 1990; Butts et al., 1993a) and the quantities of secretion vary with protein source and componsition, including the ratio of RDP to RUP (Nyachoti et al., 1997). In general, the presence of exogenous protein in the gut appears to slow the breakdown of endogenous protein (Snook and Meyer, 1964). It is possible that dietary protein not only stimulates endogenous protein secretion but also reduces digestion and absorption of endogenous protein (Nyachoti et al., 1997). Larsen et al. (2000) have demonstrated that the average duodenal flow of EN was 10.0 g/kg·DMI estimated by the difference method in lactating cows fed diets low in AA content. Ouellet et al. (2002) have found that the average duodenal flow of EN was 4.4 g/kg • DMI estimated by the ^{15}N dilution method in Holstein cows fed diets with different NDF content. Jensen et al. (2006) have reported that the duodenal flow of EN was 7.9 g/kg •DMI using the AAP method in lactating Danish Holstein-Friesian cows fed maize silage. The average duodenal flow of EN was 2.8 g/kg • DMI determined by the difference method in this study. This was close to our previous result (Zhou et al., 2008), in which the average duodenal flow of EN was 2.1 g/kg • DMI estimated by the difference method in growing goats fed diets containing different NDF levels. The differences between findings of our lab and others might result from the different computation processes among determination methods. Furthermore, the big size and the relatively large amount of protein turnover for dairy cows might result in greater losses of EN at the duodenum (Lapierre et al., 2008), which needs further examination. Larsen et al. (2001) reported that the average duodenal flow of total endogenous AA determined by the AAP method was 25.6 g/kg • DMI in dairy cows. The present average duodenal flow of total endogenous AA was 6.8 g/kg • DMI estimated by the AAP method, which was lower than our previous findings (11.8 g kg^{-1} DMI) in growing goats (Zhou et al., 2008). This difference was probably ascribed to the lower dietary NDF content in the present study (32.5% vs 35.6%), which is an important factor affecting the endogenous protein losses (Moughan et al., 1998; Hess and Seve, 1999; Ouellet et al., 2002).

Some studies have recently been conducted to determine the effects of dietary RDP or RUP on the performance, nutrients digestion and ruminal fermentation characteristics in ruminants (McCormick et al., 1999; Anderson et al., 2001; Atkinson et al., 2007). To our knowledge, few studies have been conducted to examine the effects of dietary ratio of RDP to RUP and feed intake on the flow of EN and endogenous AA in ruminants. Our results showed that there were no differences in the duodenal flow of EN and total endogenous AA within the range of dietary ratios of RDP to RUP for growing goats used in this study. However, the duodenal flow of EN and endogenous AA decreased when the ratio of RDP to RUP was decreased from 65:35 to 45:55, namely the duodenal flow of EN and endogenous AA decreased by 4.4 and 34.1, 7.0 and 18.1% for DMI1 and DMI2 treatment, respectively. Some studies showed that the endogenous losses were sensitive to the feed intake (Furuya and Kaji, 1992; Butts et al., 1993b; James et al., 2002). However, our results showed conflicting evidence for the effect of feed intake on endogenous losses. Some studies demonstrated that the endogenous losses were significantly affected by the feed intake (Butts et al., 1993b; James et al., 2002), while others showed that the feed intake did not affect the endogenous losses (Furuya and Kaji, 1992). In the present study, the duodenal flow of EN and total endogenous AA was not affected by the feed intake, but the duodenal flow of EN and total endogenous AA decreased when feed intake was decreased from 0.63 to 0.50 kg/d, namely the flow of EN and total endogenous AA at the duodenum decreased by 7.7 and 36.4%, 2.9 and 14.5% for the corresponding RDP1 and RDP2 treatment, respectively.

4.2 The ileal flow of EN and endogenous AA

There is contradictory evidence on how feed intake influences the ileal flows of EN and endogenous AA. Butts et al. (1993b) demonstrated that the ileal flows of EN and endogenous AA increased by 113 and 124% when DMI was increased from 0.90 to 2.71 kg/d in pigs. On the contrary, Furuya and Kaji (1992) found the ileal flows of EN and endogenous AA were not affected when DMI was increased by a similar magnitude in pigs. Part of these differences might be attributed due to the differences in the methodology and/or diet composition. The enzyme-hydrolyzed casein (EHC) method and regression technique of protein-free diet were applied in the studies of Butts et al. (1993b) and Furuya and Kaji (1992), respectively. We consider that the results from Butts et al. (1993b) are more reliable as their data were obtained under more physiological conditions. In the present study, the average ileal flow of EN and endogenous AA increased by 35 and 22% when DMI was increased from 0.50 to 0.63 kg/d, respectively. The differences of increased percentage for EN and endogenous AA could also be caused by the difference of increased DMI extent (201% vs 29%) and/or experimental animal (pig vs goat) when compared with the results of Butts et al. (1993b).

The ileal flow in endogenous lysine was higher for DMI2 than for DMI1, interestingly, the flow of

endogenous lysine was also affected by DMI, as observed previously (Butts et al., 1993b; Hodgkinson et al., 1999). This implies that the ileal flow of endogenous lysine was more affected by DMI. The ileal flows of EN and endogenous AA determined in the present study support the assumption that the water soluble fraction was entirely of endogenous origin. The water soluble phase had a small content of DAPA, which indicated that it had not been totally purified for bacterial cell wall. Further, enzymatically released but not absorbed protein from feed and microbes could be located in the water soluble fraction. Therefore, the different sources of protein might result in differences for individual AA content in the ileal endogenous protein.

In agreement with previous data from pigs (Rademacher et al., 1999; Pedersen and Boisen, 2002; Ravindran et al., 2004), glycine, aspartate and glutamine dominanted the AA profile of endogenous protein in the ileal digesta, while the concentration of methionine, histidine and cysteine were lower in pig ileal digesta compared with ileal digesta in the present study. The endogenous protein reaching the distal ileum was reported to consist primarily of biliary secretions and mucin glycoproteins, because these components are largely resistant to proteolysis (Moughan and Schuttert, 1991). Mucin glycoprotein is rich in aspartate and glutamine, and glycine accounts for more than 90% of the AA content of bile (Lien et al., 1997). It was also suggested that glycine, aspartate and glutamine in endogenous proteins might be reabsorbed more slowly from the gut lumen compared with other AA (Taverner et al., 1981). As a result, endogenous protein was reported to have a high content of these AA. Since mucins represented a major source of endogenous losses, one might speculate that threonine losses would be higher than those of other individual AA, and a consistent result was showed in the present study.

The flow of endogenous protein at the terminal ileum represents a balance between secretion and re-absorption. Both secretion and re-absorption can be influenced by dietary protein and peptide (Moughan and Schuttert, 1991; Nyachoti et al., 1997). Larsen et al. (2001) also demonstrated that the small intestinal re-absorption of endogenous AA showed some extremes when the duodenal flow was estimated by the difference method, and pointed out that the

Table 1. Ingredients and chemical composition of the experimental diets (% DM)

Ingredients, % of diet	Diets*	
	RDP1	RDP2
Maize stover	40.0	40.0
Ground corn	49.6	52.9
Soybean meal	7.00	-
Urea	0.30	-
Fish meal	-	4.45
$CaCO_3$	0.47	0.07
Sodium chloride	0.60	0.60
Premix†	2.00	2.00
Chemical composition		
Metabolizable energy(kJ/Kg DM)ξ	11600	11600
Crude protein	11.0	10.2
Calcium	0.37	0.37
Phosphorus	0.27	0.37
Neutral detergent fiber	32.8	32.2
RDP (%)	65.0	45.0

* RDP1, RDP:RUP = 65: 35; RDP2, RDP:RUP = 45: 55.
† Premix (/Kg) : 243.8g $MgSO_4•H_2O$, 15.8 g $FeSO_4•7H_2O$, 3.3 g $CuSO_4•5H_2O$, 13.0 g $MnSO_4•H_2O$, 14.5 g $ZnSO_4•H_2O$, 20 mg Na_2SeO_3, 60 mg KI, 40 mg $CoCl_2•6H_2O$, 28.5 mg VA, 0.44 mg VD, 12,060 mg VE.
ξ Metabolizable energy is calculated according to Zhang and Zhang (1998), other nutrient levels are measured values.

Table 2. RDP to RUP ratio and feed intake level effects on the duodenal flow, ileal flow and intestinal re-absorption of EN in goats

Item	Treatments*				SEM†	Main effect and interaction		
	RDP1		RDP2			RDP	DMI	RDP×DMI
	DMI1	DMI2	DMI1	DMI2		P		
N intake (g/d)	10.8	8.6	10.5	8.3	0.09	0.18	0.16	0.10
Duodenal flow (g/d)								
Total N	10.5	9.8	12.1	7.6	0.91	0.85	0.17	0.30
Undegraded feed N	1.44	1.59	1.87	1.43	0.07	0.61	0.57	0.18
Microbial N	7.27	6.64	8.61	5.03	0.65	0.93	0.11	0.27
Endogenous N	1.81	1.67	1.73	1.10	0.27	0.55	0.51	0.68
Ileal EN flow (g/d)	0.20	0.13	0.15	0.13	0.03	0.61	0.26	0.48
Intestinal re-absorption of EN (%)	77.4	92.7	86.5	84.5	20.63	0.50	0.42	0.93

* RDP1, RDP: RUP = 65: 35; RDP2, RDP: RUP = 45: 55; DMI1, 95% of voluntary feed intake; DMI2, 75% of voluntary feed intake.
† Standard error of means.

Table 3. RDP to RUP ratio and feed intake level effects on the flow of endogenous AA at the duodenum and ileum and intestinal re-absorption in goats

Item		Treatments*				SEM†	Main effect and interaction		
		RDP1		RDP2			RDP	DMI	RDP×DMI
		DMI1	DMI2	DMI1	DMI2		P		
Arginine	Duodenum (g/d)	0.23	0.20	0.15	0.09	0.03	0.21	0.54	0.88
	Ileum (g/d)	0.16	0.17	0.11	0.10	0.02	0.23	0.96	0.90
	Re-absorption (%)	61.4	52.4	58.7	65.8	16.08	0.53	0.25	0.95
Hisdidine	Duodenum (g/d)	0.11	0.22	0.29	0.12	0.06	0.66	0.79	0.18
	Ileum (g/d)	0.10	0.14	0.13	0.10	0.03	0.98	0.89	0.52
	Re-absorption (%)	61.8	67.4	72.1	74.1	18.08	0.86	0.97	0.11
Isoleucine	Duodenum (g/d)	0.25	0.18	0.25	0.21	0.05	0.67	0.23	0.80
	Ileum (g/d)	0.19	0.20	0.16	0.20	0.01	0.76	0.43	0.62
	Re-absorption (%)	60.1^{ab}	51.1^{b}	67.8^{a}	66.8^{b}	17.03	0.26	0.02	0.70
Leucine	Duodenum (g/d)	0.56	0.40	0.25	0.27	0.05	0.13	0.63	0.52
	Ileum (g/d)	0.32^{ab}	0.47^{a}	0.16^{b}	0.20^{ab}	0.05	0.04	0.31	0.56
	Re-absorption (%)	70.3	66.8	76.3	74.3	18.64	0.39	0.05	0.88
Lysine	Duodenum (g/d)	0.37	0.32	0.25	0.45	0.06	0.99	0.54	0.32
	Ileum (g/d)	0.22^{ab}	0.32^{a}	0.12^{b}	0.30^{ab}	0.03	0.27	0.03	0.51
	Re-absorption (%)	67.8^{a}	57.5^{b}	71.8^{a}	74.9^{ab}	18.20	0.14	0.03	0.39
Phenylalanine	Duodenum (g/d)	0.44^{ab}	0.60^{a}	0.17^{b}	0.26^{ab}	0.07	0.05	0.39	0.81
	Ileum (g/d)	0.20^{b}	0.57^{a}	0.12^{b}	0.20^{b}	0.06	0.03	0.02	0.12
	Re-absorption (%)	57.8	46.2	58.7	54.5	23.80	0.50	0.08	0.28
Methionine	Duodenum (g/d)	0.05	0.03	0.04	0.03	0.01	0.99	0.58	0.91
	Ileum (g/d)	0.02	0.03	0.02	0.03	0.01	0.71	0.36	0.90
	Re-absorption (%)	62.5	53.8	69.1	69.2	19.58	0.56	0.16	0.72
Threonine	Duodenum (g/d)	0.23	0.33	0.32	0.23	0.06	0.97	0.96	0.52
	Ileum (g/d)	0.31	0.38	0.34	0.38	0.08	0.95	0.75	0.93
	Re-absorption (%)	39.4^{a}	37.3^{a}	45.5^{a}	47.1^{b}	13.41	0.12	0.16	0.03
Valine	Duodenum (g/d)	0.27	0.24	0.46	0.25	0.05	0.47	0.39	0.51
	Ileum (g/d)	0.13	0.26	0.22	0.21	0.03	0.71	0.38	0.34
	Re-absorption (%)	65.6	62.9	65.9	60.1	22.71	0.96	0.06	0.63
Alanine	Duodenum (g/d)	0.25	0.22	0.20	0.14	0.03	0.45	0.56	0.82
	Ileum (g/d)	0.18	0.28	0.15	0.14	0.04	0.28	0.59	0.48
	Re-absorption (%)	60.2	53.4	65.7	68.6	20.64	0.24	0.11	0.47
Glycine	Duodenum (g/d)	0.32	0.23	0.37	0.33	0.08	0.45	0.47	0.81
	Ileum (g/d)	0.34	0.27	0.42	0.39	0.06	0.45	0.71	0.87
	Re-absorption (%)	39.8	24.9	36.1	44.7	13.01	0.57	0.07	0.49
Tyrosine	Duodenum (g/d)	0.24	0.19	0.18	0.16	0.04	0.66	0.70	0.86
	Ileum (g/d)	0.14	0.12	0.06	0.12	0.02	0.44	0.57	0.45
	Re-absorption (%)	55.5^{ab}	57.1^{a}	81.8^{a}	53.6^{b}	23.18	0.74	0.03	0.01
Aspartate	Duodenum (g/d)	0.24	0.49	0.26	0.24	0.06	0.16	0.16	0.11
	Ileum (g/d)	0.33	0.64	0.36	0.30	0.06	0.23	0.33	0.18
	Re-absorption (%)	45.5	40.9	35.3	46.8	10.18	0.39	0.52	0.44
Cysteine	Duodenum (g/d)	0.008	0.009	0.005	0.006	0.01	0.10	0.23	0.99
	Ileum (g/d)	0.007^{ab}	0.011^{a}	0.004^{b}	0.005^{b}	0.01	0.02	0.20	0.33
	Re-absorption (%)	44.7	38.0	58.0	50.9	19.58	0.17	0.13	0.87
Serine	Duodenum (g/d)	0.36	0.21	0.36	0.34	0.04	0.64	0.57	0.63
	Ileum (g/d)	0.37	0.30	0.23	0.34	0.05	0.64	0.86	0.45
	Re-absorption (%)	47.3	46.9	62.8	47.1	24.75	0.53	0.13	0.82
Glutamine	Duodenum (g/d)	0.21	0.16	0.32	0.18	0.09	0.53	0.37	0.72
	Ileum (g/d)	0.27	0.27	0.46	0.30	0.08	0.53	0.63	0.65
	Re-absorption (%)	46.6	31.5	39.2	54.5	17.06	0.18	0.47	0.08
Total	Duodenum (g/d)	4.16	4.04	3.87	3.31	0.53	0.82	0.75	0.68
	Ileum (g/d)	2.34	1.92	2.74	2.24	0.30	0.27	0.08	0.35
	Re-absorption (%)	77.0	77.2	80.0	77.0	18.26	0.98	0.09	0.81

* RDP1, RDP: RUP = 65: 35; RDP2, RDP: RUP = 45: 55; DMI1, 95% of voluntary feed intake; DMI2, 75% of voluntary feed intake.

† Standard error of means.

a, b Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

Re-absorption of individual endogenous AA was very variable and related to the secretion of digestive juice with specific AA composition. The difference of re-absorption in endogenous isoleucine, leucine, lysine and tyrosine might result from the different flows at the duodenum and ileum. The average apparent re-absorption of AA in the intestine ranged from 62.3 to 82.5% in sheep (Zebrowska and Kowalczyk, 1991; Lammers-Wienhoven et al., 1997). The average re-absorption of endogenous AA (58.9%) was lower than the reported values in the present study, and the differences might be ascribed to different experimental animal and diet composition.

5. Conclusions

This study demonstrated that the duodenal and ileal flows of EN and total endogenous AA were not affected by the dietary ratio of RDP to RUP and feed intake, while the duodenal flow of EN and total endogenous AA decreased when the DMI and the ratio of RDP to RUP decreased, and the ileal flow of total endogenous AA decreased when DMI decreased, but it increased when the dietary ratio of RDP to RUP decreased in goats.

Acknowledgements

The authors wish to acknowledge the financial support received from National Natural Science Foundation of China (No.31001024, No. 31320103917), Ministry of Science and Technology of the People`s Republic of China (No. 2012BAD14B17), Chinese Academy of Sciences (No. KFJ-EW-STS-071) and Hunan Provincial Science and Technology Department (2013NK4003).

This content has not been published or submitted for publication elsewhere.

We acknowledge Prof. Zhi-liang Tan for provide assistance of experiment design and paper revision. All the authors state that there are no conflicts of interest.

Corresponding author:
Zhiliang Tan, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha PO Box 10, Hunan 410125, P. R. China.
Tel.: +86 731 84619702; Fax: +86 731 84612685;
E-mail address: zltan@isa.ac.cn.

Running head:
Endogenous nitrogen and amino acids flow

References

1. Anderson, L.P., Paterson, J.A., Ansotegui, R.P., Cecava, M., & Schmutz, W., (2001). The effects of degradable and undegradable intake protein on the performance of lactating first-calf herfers. *Journal of Animal Science, 79,* 2224-2232.
2. AOAC, (2002). Association of Official Analytical Chemists: Official Methods of Analysis, 16th Edition., Washington, D.C.
3. Atkinson, R.L., Toone, C.D., & Ludden, P.A., (2007). Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on site and extent of digestion and ruminal characteristics in lambs fed low-quality forage. *Journal of Animal Science, 85,* 3322-3330.
4. Bunting, L.D., Boling, J.A., MacKown, C.T., & Davenport, G.M., (1989). Effect of dietary protein level on nitrogen metabolism in the growing bovine: II. Diffusion into and utilization of endogenous urea nitrogen in the rumen. *Journal of Animal Science, 67,* 820-826.
5. Butts, C.A., Moughan, P.J., Smith, W.C., & Carr, D.H., (1993a). Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg body weight): The effect of protein-free, synthetic amino acid, peptide and protein alimentation. *Journal of the Science of Food and Agriculture, 61,* 31-40.
6. Butts, C.A., Moughan, P.J., Smith, W.C., eynolds, G.W., & Garrick, D.J., (1993b). The effect of food dry matter intake on the endogenous ileal amino acid extraction determined under peptide alimentation in the 50 kg liveweight pig. *Journal of the Science of Food and Agriculture, 62,* 235-243.
7. Darragh, A.J., Moughan, P.J., & Smith, W.C. (1990). The effect of amino acid and peptide alimentation on the determination of endogenous amino acid flow at the terminal of rat. *Journal of the Science of Food and Agriculture, 51,* 47-56.
8. Egan, A.R., Boda, K., & Varady, J., (1986). Regulation of nitrogen metabolism and recycling. In Control of Digestion and Metabolisms in Ruminants. p. 386-402. *In*: Milligan, L.P.; Grovum, W. L.; Dobson, A., eds. Prentice Hall, Englewood Cliffs, New Jersey.
9. Evans, R.A., Axford, R.F., & Offer, N.W. (1975). A method for estimating the quantities of microbial and dietary proteins flowing in the duodenal digesta of ruminants. *Proceedings of the Nutrition Society, 34,* 65A-66A.
10. Furuya, S., & Kaji, Y., (1992). The effects of feed intake and purified cellulose on the endogenous ileal amino acid flow in growing pigs. *British Journal of Nutrition, 68,* 463-472.
11. Hansen, B., (1989). Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agriculturae Scandinavica. *Section A: Animal Science, 39,* 113-118.
12. Hess, V., & Seve, B. (1999). Effect of body weight and feed intake level on basal ileal

endogenous losses in growing pigs. *Journal of Animal Science, 77,* 3281-3288.
13. Hess, V., Ganier, P., Thibault, J.N., & Seve, B. (2000). Comparison of the isotope dilution method for determination of the ileal endogenous amino acid losses with labelled diet and labelled pigs. *British Journal of Nutrition, 83,* 123-130.
14. Hodgkinson, S.M., Souffrant, W.B., & Moughan, P.J., (1999). Comparison of three methods for determining endogenous ileal protein flow in the growing pig. In Manipulating Pig Production VII. p. 270. *In*: Cranwell, P.D., eds. Canberra: Australian Pig Science Association.
15. Hutton, K., Bailey, F.J. & Annison, E.F. (1971). Measurement of the bacterial nitrogen entering the duodenum of ruminant using diaminopimelic acid as a marker. *British Journal of Nutrition, 25,* 165-173.
16. James, K.A.C., Butts, C.A., Koolaard, J.P., Donaldson, H.E., Scott, M.F., & Moughan, P.J., (2002). The effect of food dry matter intake on the flow of amino acids at the terminal ileum for rats fed an enzyme-hydrolysed casein-based diet. *Journal of the Science of Food and Agriculture, 82,* 1128-1135.
17. Jensen, C., Weisbjerg, M.R., & Hvelplund, T., (2006). Evaluation of methods for estimating the amino acid supply to the duodenum of microbial, endogenous and undegraded feed protein on maize silage diets fed to dairy cows. *Animal Feed Science and Technology, 131,* 1-24.
18. Lammers-Wienhoven, S.C., Voigt, W.J., Ram, L., Bruchem, J.V., Ketelaars, J., Tamminga, S., & Van Bruchem, J., (1997). Effect of cell walls, dry matter and protein supply on endogenous nitrogen flow in the small intestine of sheep. *Journal of Animal Physiology and Animal Nutrition, 78,* 129-140.
19. Lapierre, H., Ouellet, D.R., Berthiaume, R., Martineau, R., Holtrop, G., & Lobley, G.E., (2008). Distribution of ^{15}N in amino acids during ^{15}N-leucine infusion: impact on the estimation of endogenous flows in dairy cows. *Journal of Dairy Science, 91,* 2702-2714.
20. Larsen, M., Madsen, T.G., Weisbjerg, M.R., Hvelplund, T., & Madsen, J., (2000). Endogenous amino acid flow in the duodenum of dairy cows. *Acta Agriculturae Scandinavica. Section A: Animal Science, 50,* 161-173.
21. Larsen, M., Madsen, T.G., Weisbjerg, M.R., Hvelplund, T., & Madsen, J., (2001). Small intestinal digestibility of microbial and endogenous amino acids in dairy cows. *Journal of Animal Physiology and Animal Nutrition, 85,* 9-21.
22. Lien, K.A., Sauer, W.C., & Fenton, M., (1997). Mucin output in ileal digesta of pigs fed a protein-free diet. *Zeitschrift für Ernährungswissenschaft, 36,* 182-190.
23. Ludden, P.A., & Kerley, M.S. (1997). Amino acid and energy interrelationship in growing beef steers: I. The effect of level of feed intake on ruminal characteristics and intestinal amino acid flows. *Journal of Animal Science, 75,* 2550-2560.
24. Mason, V.C., Bech-Andersen, S., & Rudemo, M. (1980). Hydrolysate preparation for amino acid determinations in feed constituents. 8. Studies of oxidation conditions for streamlined procedures. *Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde, 43,* 146-164.
25. McCormick, M.E., French, D.D., Brown, T.F., Cuomo, G.J., Chapa, A.M., Fernandez, J.M., Beatty, J.F., & Blouin, D.C. (1999). Crude protein and rumen undegradable protein effects on reproduction and lactation performance of Holstein cows. *Journal of Dairy Science, 82,* 2697-2708.
26. Moughan, P.J., & Schuttert, G. (1991). Composition of nitrogen-containing fractions in digesta from the distal ileum of pigs fed a protein-free diet. *Journal of Nutrition, 121,* 1570-1574.
27. Moughan, P.J., Souffrant, W.B., & Hodgkinson, S.M. (1998). Physiological approaches to determining gut endogenous amino acid flows in the mammal. *Archiv für Tierernaehrung, 51,* 237-252.
28. NRC, (2001). Protein and amino acids. In Nutrient Requirements of Dairy Cattle, 7th ed., pp. 43-104. National Research Council, Washington, D.C.
29. Nyachoti, C.M., de Lange, C.F.M., McBride, B.W., & Schulze, H., (1997). Significance of endogenous gut protein losses in the nutrition of growing pigs. *Canada Journal of Animal Science, 77,* 149-163.
30. Øskov, E.R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agriculture Science, 92,* 499-503.
31. Ouellet, D.R., Demers, M., Zurr, G.; Lobley, G.E., Seoane, J.R., Nolan, J.V., & Lapierre, H. (2002). Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. *Journal of Dairy Science, 85,* 3013-3025.
32. Pedersen, C., & Boisen, S. (2002). Establishment of tabulated values for standardized ileal digestibility of crude protein and essential amino acids in common feedstuffs for pigs. Acta Agriculturae Scandinavica. *Section A: Animal Science, 52,* 121-140.

33. Powell, M.J.D., (1964). An efficient method for finding the minimum of a function of several variables without calculating derivatives. *Computer Journal, 7,* 155-162.
34. Rademacher, M., Sauer, W.C., & Jansman, A.J.M., (1999). Standardized Ileal Digestibility of Amino Acids in Pigs. Hanua: Feed Additivies Division, AG Degussa-Huis.
35. Ravindran, V., Hew, L.I., Ravindran, G., & Bryden, W.L., (2004). Endogenous amino acid flow in the avain ileum: quantification using three techniques. *British Journal of Nutrition, 92,* 217-223.
36. Reed, J.J., Lardyl, G.P., Bauer, M.L., Gilbery, T.C., & Caton, J.S., (2004). Effect of field pea level on intake, digestion, microbial efficiency, ruminal fermentation and in situ disappearance in beef steers fed growing diets. *Journal of Animal Science, 82,* 2123-2130.
37. SAS, (1996). SAS User's Guide: Statistics, Version 6.12 Edition. SAS Institute Inc., Cary, N.C.
38. Schneeman, B., (1982). Digestive enzyme activities from the pancreas in response to diet. In Physiologie digestive chezle porc. p. 125-131 *In*: Laplace, J. P., Corring, T., Rérat, A., eds. Le Séminaire Int. 27-29 October 1982. Jouy-en-Josas-Versailles, France.
39. Schurch, A.F., Lloyd, L.E., & Crampton, E.W., (1950). The use of chromic oxide as an index for determining the digestibility of a diet. *Journal of Nutrition, 41,* 629-636.
40. Sève, B., & Hess, V., (2000). Amino acid digestibility in formulation of diets for pigs: present interest and limitations, future prospects. In Recent advances in animal nutrition. p. 167-181. *In*: Garnsworthy, P. C., Wiseman, J., eds. Nottingham University Press, Nottingham.
41. Simon, O., Zebrowska, T., Bergner, H., Münchmeyer, R., (1983). Investigations on the pancreatic and stomach secretion in pigs by means of continuous infusion of ^{14}C-amino acids. *Archievs of Animal Nutrition, 33,* 9-22.
42. Snook, J.T., & Meyer, J.H., (1964). Response of digestive enzymes to dietary protein. *Journal of Nutrition, 82,* 409-414.
43. Sun, Z.H., Tan, Z.L., Yao, J.H., Tang, Z.R., Shan, J.G., Hu, J.P., Tang, S.X., & Jiang, Y.M., (2007). Effects of intra-duodenal provision of limiting amino acids on serum concentrations of immunoglobulins and tissue concentrations of DNA and RNA in growing goats fed a maize stover-based diet. *Small Ruminant Research, 69,* 159-166.
44. Tamminga, S., Schulze, H., Van Bruchem, J., & Huisman, J., (1995). The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. *Archiv für Tierernaehrung, 48,* 9-22.
45. Tan, Z.L., Lu, D.X., Hu, M., Niu, W.Y., Han, C.Y., Ren, X.P., Na, R., & Lin, S.L., (2001). Effects of dietary nitrogen sources on fiber digestion and ruminal fluid characteristics in sheep fed wheat straw. *Asian-Australasian Journal of Animal Science, 14,* 1374-1382.
46. Taverner, M.R., Hume, I.D., & Farrell, D.J., (1981). Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. *British Journal of Nutrition, 46,* 149-158.
47. van Bruchem, J., Voigt, J., & Lammers-Wienhoven, S.C.W., (1997). Secretion and reabsorption of endogenous protein along the small intestine of sheep: estimates derived from ^{15}N dilution of plasma non-protein-N. *British Journal of Nutrition, 77,* 273-286.
48. Zebrowska, T., & Kowalczyk, J., (1991). Nitrogen secretion into isolated loops of the small intestine in conscious sheep. *Journal of Animal Physiology and Animal Nutrition, 65,* 133-139.
49. Zhang, H.F., & Zhang, Z.Y., (1998). Animal Nutrition Parameters and Feeding Standard.China Agriculture Press, Beijing, China.
50. Zhao, X.G., Wang, M., Tan, Z.L., Tang, S.X., Sun, Z.H., Zhou, C.S., & Han, X.F., (2009). Effects of rice straw particle size on chewing activity, feed intake, rumen fermentation and digestion in goats. Asian-Australas. *Journal of Animal Science, 22, 1256-1266.*
51. Zhou, C.S., Jiang, H.L., Tan, Z.L., Zhao, X.G., Sun, Z.H., Tang, S.X., & Wang, M., (2008). Estimation of endogenous nitrogen and amino acids flow at the duodenum and ileum in growing goats fed on different NDF level diets. *Journal of Applied Animal Research, 33,* 137-144.

8/3/2014