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Effects of Non-protein Energy Intake on Whole Body Protein Synthesis, Nitrogen Retention and Glucose Turnover in Goats

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ABSTRACT : The responses of whole body protein and glucose kinetics and of nitrogen (N) metabolism to non-protein energy intake (NPEI) were determined using an isotope dilution approach and measurement of N balance in three adult male goats. The diets containing 1.0, 1.5 and 2.0 times ME maintenance requirement, with fixed intake of CP (1.5 times maintenance) and percentage of hay (33%), were fed twice daily for each 21 d experimental period. After an adaptation period of 11 d, N balance was determined over 3 d. On day 17, whole body protein synthesis (WBPS) and glucose irreversible loss rate (ILR) were determined during the absorptive state by a primed-continuous infusion of [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine, [$^2H_4$]tyrosine and [$^{13}C_6$]glucose, with simultaneous measurements of plasma concentrations of metabolites and insulin. Ruminal characteristics were also measured at 6 h after feeding over 3 d. Nitrogen retention tended to increase ($p<0.10$) with increasing NPEI, although digestible N decreased linearly ($p<0.05$). Increasing NPEI decreased ($p<0.01$) ammonia N concentration, but increased acetate ($p<0.05$) and propionate ($p<0.05$) concentrations in the rumen. Despite decreased plasma urea N concentration ($p<0.01$), increased plasma tyrosine concentration ($p<0.05$), and trends toward increased plasma total amino N ($p<0.10$) and phenylalanine concentrations ($p<0.10$) were found in response to increasing NPEI. Increasing NPEI increased ILR of both glucose ($p<0.01$) and phenylalanine ($p<0.05$), but did not affect ($p\geq0.10$) that of tyrosine. Whole body protein synthesis increased ($p<0.05$) in response to increasing NPEI, resulting from increased utilization rate for protein synthesis ($p<0.05$) and unchanged hydroxylation rate of phenylalanine ($p\geq0.10$). These results suggest that increasing NPEI may enhance WBPS and glucose turnover at the absorptive state and improve the efficiency of digestible N retention in goats, with possibly decreased ammonia and increased amino acid absorption. In addition, simultaneous increases in WBPS and glucose ILR suggest stimulatory effect of glucose availability on WBPS, especially when sufficient amino acid is supplied. (**Key Words :** Protein Synthesis, Phenylalanine Metabolism, Glucose Metabolism, Energy Intake, Stable Isotope, Goat)

INTRODUCTION

In ruminants, dietary non-protein energy, which is defined as the dietary energy attributed to ingredients other than crude protein (CP), has a profound influence not only on the composition and amount of the amino acids absorbed due to effects on microbial fermentation in the rumen, but also on the metabolic fate of amino acids in the body through changing supply of energy substrates (Asplund, 1994). However, there are a few studies that have examined the effect of non-protein energy independently of CP, and the results obtained are inconsistent. Nissen and Ostaszeuski (1985) reported that supplemental energy as starch decreased leucine flux, utilization for protein synthesis and oxidation of leucine beyond 17 h after feeding in sheep fed twice daily. In contrast, Fujita et al. (2006a) showed that starch supplementation enhanced whole body protein synthesis (WBPS) during 5 to 7 h after feeding in goats fed twice daily. These previous results may show that the effects of non-protein energy intake (NPEI) on whole body protein kinetics are different depending on metabolic state related to feeding.

Since these previous results were obtained at different metabolic states under supplementation of a refined starch as a non-protein energy source, further study is needed to confirm the effect of NPEI on whole body protein kinetics and the relationship between responses of WBPS and glucose kinetics to increasing NPEI at the absorptive and/or post-absorptive states under other feeding conditions.

The present study was designed to investigate the effects of NPEI on whole body kinetics of protein and glucose at the absorptive state in adult male goats fed twice daily under conditions of changing amounts of conventional

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Table 1. The amounts of diets fed to goats in each dietary treatment

Treatment[1]	The amounts of diets fed (g/kg BW/d)		
	Mixed hay	Ground corn	Ground soybean meal
1.0×M	5.49	7.47	3.52
1.5×M	8.24	14.60	1.86
2.0×M	10.98	21.74	0.20

[1] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than crude protein.

feeds and holding a constant ratio of roughage to concentrate in the diets. The kinetics were determined by isotope dilution approaches with [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine and [$^2H_4$]tyrosine for protein, and [$^{13}C_6$]glucose for glucose.

MATERIALS AND METHODS

Animals and management

Three Saanen intact male goats (3 yr, initial body weight (BW) 37.8±6.4 kg; mean±SE) were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery. Each animal was kept individually in a metabolism cage in a controlled environment chamber at air temperature of 20±1°C. Mixed hay (60% of reed canarygrass and 40% of orchardgrass; metabolizable energy (ME) 1.73 Mcal/kg, CP 10.6%), ground corn (ME 2.78 Mcal/kg, CP 7.3%) and ground soybean meal (ME 2.77 Mcal/kg, CP 49.0%) were used as experimental diets. Each animal was offered three levels of ME, which were 1.0 (1.0×M), 1.5 (1.5×M) and 2.0 (2.0×M) times maintenance requirement (National Research Council, 1985), by changing the amount of each experimental diet (Table 1). Crude protein intake and the percentage of mixed hay intake in each dietary treatment were held at levels of 1.5 times maintenance requirement (National Research Council, 1985) and 33%, respectively, so that different ME and the same CP intakes in each treatment allowed different levels of NPEI. The experimental period for each dietary treatment was 21 d. Animals were weighed in the morning of day 1 and 16 of each period. One animal was allotted to ascending order of energy intake and the others to descending order. The diets were equally divided into two meals and fed to the animals at 08:30 and 20:30 h. Water and mineral blocks were available with free access. Animals completely ate the diet within half an hour after feeding. Surgery, management, and blood sampling were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Rumen fluid sampling

On day18, 19 and 20 of each dietary treatment, 50 ml of rumen fluid were taken at 6 h after feeding via a stomach tube inserted orally into the rumen. The pH of the ruminal fluid was immediately measured with a pH-meter (HM-10P, Toa Electronics Ltd., Japan), and then the liquid fraction was separated by centrifuging at 1,500 g for 15 min. A fraction (1 ml) was mixed with 1 ml of 0.4 N HCl to fix free ammonia for ruminal ammonia nitrogen (N) analysis. This sample and 5 ml of the residual liquid fraction, for ruminal volatile fatty acids (VFA) measurement, were stored at -20°C until each analysis.

Urine and feces collections

Over 3 d between day 13 and 15 of each dietary treatment, urine and feces were collected separately through a 3 mm plastic screen as a separator once daily for N balance measurement. Urine was collected into a bottle containing 100 ml of 3 N H_2SO_4 and the volume was recorded. A subsample of urine (50 ml) was stored at -20°C for N, urea N and creatinine analyses. Feces were dried at 60°C for 1 d and then weighed after placing in a room atmosphere over 5 d. The samples of diet and feces were ground through a 1 mm screen and the subsamples were then stored at room temperature until N analysis.

Isotope dilution experiment

On day 17 of each dietary treatment, the isotope dilution experiment was conducted to determine whole body glucose and protein kinetics over 4 h between 3 and 7 h after feeding using a primed-continuous infusion method. A catheter for infusion was inserted into a jugular vein on the day before the experiment, and another for blood sampling was inserted into the carotid artery within the skin loop at least 2 h before initiation of the experiment. Both catheters were filled with a sterile solution of trisodium citrate (38 g /L). [$^2H_5$]Phenylalanine (L-phenyl-d5-alanine, 99 atom% D excess, Isotec, USA), [$^2H_2$]tyrosine (L-hydroxyphenyl-3, 5-d2-alanine, 98 atom% D excess, Isotec, USA), [$^2H_4$]tyrosine (L-hydroxyphenyl-2, 3, 5, 6-d4-alanine, 98 atom% D excess, Isotec, USA), and [$^{13}C_6$]glucose (D-glucose-$^{13}C_6$, 99 atom% ^{13}C excess, Isotec, USA) were dissolved in sterile saline to prepare tracer solutions for simultaneous measurement of protein and glucose kinetics. After collection of a background blood sample (12 ml), a priming injection of [$^2H_5$]phenylalanine (0.750 mg/kg BW), [$^2H_2$]tyrosine (0.375 mg/kg BW), [$^2H_4$]tyrosine (0.120 mg/kg BW) and [$^{13}C_6$]glucose (0.225 mg/kg BW) was administered with a syringe through the infusion catheter over 1 min. Thereafter, a continuous infusion of [$^2H_5$]phenylalanine (0.750 mg/kg BW/h), [$^2H_2$]tyrosine (0.370 mg/kg BW/h) and [$^{13}C_6$]glucose (0.180 mg/kg BW/h) was initiated using a peristaltic pump (AC-2120, Atto Co. Ltd., Japan). Blood (12 ml) was taken every 30 min during the last 2 h of the infusion period, which corresponded to a period from 5 to 7

Table 2. The effects of non-protein energy intake on nitrogen balance in adult goats[1]

	1.0×M[2]	1.5×M	2.0×M	Significance
n	3	3	2	
Urinary creatinine excretion (g/kg BW/d)	0.025±0.001	0.023±0.001	0.025±0.001	NS
Urinary urea nitrogen excretion (g/kg BW/d)	0.287±0.017[a]	0.179±0.017[b]	0.166±0.023[b]	*
Nitrogen balance (g/kg BW/d)				
Intake	0.466±0.002	0.463±0.002	0.468±0.002	NS
Excretion				
Urine	0.319±0.015[a]	0.215±0.015[b]	0.199±0.020[b]	*
Feces	0.080±0.007[b]	0.130±0.007[a]	0.169±0.009[a]	**
Total	0.399±0.009	0.345±0.009	0.368±0.012	#
Retention	0.067±0.010	0.118±0.010	0.100±0.013	#
Apparent digestible nitrogen (g/kg BW/d)	0.387±0.008[a]	0.333±0.008[b]	0.298±0.010[b]	*
Apparent nitrogen digestibility (%)	82.9±1.5[a]	71.9±1.5[b]	63.9±2.0[b]	**
Ratio of nitrogen retention				
To nitrogen intake	0.145±0.021	0.255±0.021	0.213±0.027	#
To apparent digestible nitrogen	0.171±0.034	0.353±0.034	0.341±0.045	#

[1] Values represent least squares means±SE of two or three animals.

[2] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than crude protein.

[a, b] Different superscripts within a row are significantly different at $p<0.05$.

NS: Not significant. # $p<0.10$. * $p<0.05$, ** $p<0.01$.

h after feeding. The blood samples were placed in heparinized tubes and stored in crushed ice until centrifugation. The infusion rate of tracer solution was recorded every 30 min throughout the experiment. Plasma was separated from the blood by centrifuging at 8,000 g for 10 min and stored at -20°C until analyses of plasma concentrations of metabolites and insulin, and plasma enrichments of phenylalanine, tyrosine and glucose.

Analysis

The N contents of the diets, urine and feces were determined by a colorimetric method for ammonia N (Weatherburn, 1967) after Kjeldahl digestion. Ammonia N in ruminal fluid was determined by the same colorimetric method. Individual ruminal VFA concentrations were measured by gas chromatography (HP5890A, Hewlett Packard, Avondale, USA) after deproteinization by adding 0.5 ml of metaphosphoric acid solution (100 g/L) and 0.5 ml of 30 mM crotonic acid solution as an external standard to 1 ml of the ruminal liquid fraction. Urine creatinine concentration was measured by a colorimetric method (Taussky, 1956). Plasma and urine urea N concentrations were determined by a modified diacetylmonoxim method (Coulombe and Favreau, 1963). Plasma glucose concentration was measured with an automated glucose analyzer (Model GLU-1, Erma Optical Works Ltd., Tokyo). Plasma concentrations of total amino N and lactate were determined by a dinitrophenol method (Rapp, 1963) and as described by Taylor (1996), respectively. Plasma free fatty acids (FFA) concentration was determined using a diagnostic kit (NEFA-C test wako, Wako, Japan). Plasma insulin concentration was measured with a radioimmunoassay kit (IRI 'Eiken', Eiken Chemical, Japan).

Plasma concentrations of phenylalanine and tyrosine, and plasma enrichments of these amino acids and glucose were determined as described by Fujita et al. (2006a). Briefly, plasma (1 ml) was mixed with 0.1 ml of a solution of external standards (0.5 mmol/L each) for measurement of phenylalanine (m-fluoro-phenylalanine) and tyrosine (3-fluoro-tyrosine) concentrations and then deproteinized by adding 1 ml of sulufosalicylic acid solution (40 g/L). Subsequently, the supernatant was applied to a dual column consisting of 0.5 ml of cation exchange resin (Dowex 50 W×8, hydrogen form) and 1.5 ml of anion exchange resin (Dowex 1×8, acetate form), and then the column was washed with distilled water. A glucose fraction was obtained by collecting the resulting eluent, and an amino acid fraction by eluting from the cation exchange resin with 4 M NH_4OH. After drying of the fractions, glucose and the amino acids were derivatized to their aldononitrilepentacetate and t-butyldimethylsilyl derivatives, respectively. Concentrations of phenylalanine and tyrosine, and enrichments (mol% excess) of [$^{13}C_6$]glucose, [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine and [$^2H_4$]tyrosine were measured by an electron impact ionization-selected ion monitoring method using a gas chromatograph-mass spectrometer (G-3000 gas-chromatograph-M-2000 mass spectrometer, Hitachi, Japan). The following ions were monitored: m/z 336, 341 and 354 for phenylalanine, [$^2H_5$]phenylalanine and m-fluoro-phenylalanine, and m/z 466, 468, 470 and 484 for tyrosine, [$^2H_2$]tyrosine, [$^2H_4$]tyrosine and 3-fluoro-tyrosine, respectively.

Table 3. The effects of non-protein energy intake on ruminal characteristics at 6 h after feeding in adult goats[1]

	1.0×M[2]	1.5×M	2.0×M	Significance
n	3	3	2	
pH	6.48±0.02[a]	6.36±0.02[b]	6.50±0.02[a]	*
Ammonia nitrogen (mg/dl)	22.7±1.3[a]	10.0±1.3[b]	4.7±1.7[b]	**
Volatile fatty acids (mM)				
Total	42.0±1.3[b]	56.1±1.3[a]	52.1±1.7[a]	**
Acetate	28.9±1.3[b]	38.7±1.3[a]	35.4±1.7[a]	*
Propionate	6.4±0.5[b]	9.3±0.5[ab]	11.3±0.7[a]	*
Isobutyrate	0.7±< 0.1[a]	0.6±<0.1[ab]	0.5±<0.1[b]	*
n-Butyrate	4.7±0.7	5.9±0.7	3.4±0.9	NS
Isovalerate	0.6±0.3	0.9±0.3	0.9±0.3	NS
n-Valerate	0.4±0.1	0.7±0.1	0.6±0.1	NS

[1] Values represent least squares means±SE of two or three animals.

[2] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than crude protein.

[a,b] Different superscripts within a row are significantly different at $p<0.05$.

NS: Not significant, * $p<0.05$, ** $p<0.01$.

Calculations

The data for ruminal characteristics, fecal and urinary components, and N retention and digestibility were obtained as averaged values for 3 d in each animal. The data for the concentrations of plasma components and the enrichments of glucose, phenylalanine and tyrosine in the isotope dilution experiment were obtained as averaged values of five samples during the last 2 h of the infusion period.

The whole body irreversible loss rates (ILR; Q) of glucose, phenylalanine and tyrosine, the rate of phenylalanine hydroxylation (the rate of phenylalanine conversion to tyrosine; Q_{pt}) and the utilization rate for protein synthesis of phenylalanine (S_p) were derived as described previously (Fujita et al., 2006a).

$$Q = I\times(E_i/E_p-1)$$

$$Q_{pt} = Q_{tyr}\times(E_{tyr}/E_{phe})\times(Q_{phe}/(I_{phe}+Q_{phe}))$$

$$S_{phe} = Q_{phe}-Q_{pt}$$

where I is the infusion rate of [$^{13}C_6$]glucose, [$^2H_5$]phenylalanine and [$^2H_2$]tyrosine, E_i and E_p are enrichments of these tracers in tracer solutions and plasma, respectively. Q_{phe} and Q_{tyr} are the ILR of phenylalanine and tyrosine, respectively. E_{tyr} and E_{phe} are the respective enrichments of [$^2H_4$]tyrosine and [$^2H_5$]phenylalanine in plasma, and I_{phe} is the infusion rate of [$^2H_5$]phenylalanine. Whole body protein synthesis was calculated by assuming that the phenylalanine content of protein is 0.0333 g/g CP in goats (Smith, 1980).

Statistical analysis

All data were analyzed by a split-plot design with a group (ascending or descending order of energy intake) as a main-plot and energy intake as a sub-plot using a General Linear Model procedure of SAS (1996). No significant group effects were detected in any data ($p>0.05$), which were considered as no effect of order of energy intake in the present study. The consideration of significant effect of energy intake was at $p<0.05$, and that of tendency was at $0.05\leq p<0.10$. When the effect was significant, a comparison between each energy intake level was conducted at $p<0.05$ by Tukey-Kramer method.

RESULTS

Only two sheep were used for 2.0×M, because one of the animals refused feed. A reason for the refusal of feed in the animal is unknown; the amount of feed given may have been too large for the animal, since the animal looked healthy, except for refusing feed, and again ate it with its residue after a few days. Thus, least squares means and their SE for all measurements are shown in tables, obtained from the ANOVA with SAS. Furthermore, the data for N balance were expressed as g/kg BW/d, since the animal excluded for 2.0×M was heavier than the others.

Although urinary creatinine excretion was unaffected ($p\geq0.10$) by NPEI, urinary urea N and N excretion decreased ($p<0.05$) with increasing NPEI (Table 2). An increase in fecal N excretion ($p<0.01$) in proportion to NPEI resulted in decreases in apparent digestible N ($p<0.05$) and apparent N digestibility ($p<0.01$). A trend toward reduction in total N excretion ($p<0.10$) produced a trend toward increase in N retention ($p<0.10$) in response to increasing NPEI because of constant N intake ($p\geq0.10$) for any dietary treatment. In consequence, the ratios of N retention to N intake and digestible N tended to increase ($p<0.10$) as NPEI increased.

At 6 h after feeding, ruminal pH was lower ($p<0.05$) for 1.5×M than 1.0×M and 2.0×M (Table 3). Increasing NPEI

Table 4. The effects of non-protein energy intake on the concentrations of plasma metabolites and insulin, the kinetics of plasma glucose, phenylalanine and tyrosine, and whole body protein synthesis (WBPS) during 2 h from 5 to 7 h after feeding in adult goats[1]

	1.0×M[2]	1.5×M	2.0×M	Significance
n	3	3	2	
Plasma concentrations				
Glucose (mg/dl)	65±1	59±1	66±2	#
Lactate (mg/dl)	5.38±0.71	5.11±0.71	5.98±0.94	NS
Free fatty acids (μEq/L)	106±18	93±18	39±23	NS
Urea nitrogen (mg/dl)	16.9±0.3[a]	11.7±0.3[b]	7.3±0.3[c]	**
Total amino nitrogen (mg/dl)	4.19±0.25	4.76±0.25	5.87±0.33	#
Phenylalanine (μM)	46.4±3.9	53.7±3.9	70.7±5.1	#
Tyrosine (μM)	69.8±5.0[b]	91.3±5.0[ab]	114.3±6.6[a]	*
Insulin (μU/ml)	13.9±0.6	14.2±0.6	17.7±0.8	#
Irreversible loss rates				
Glucose (mg/kg BW/min)	1.75±0.06[c]	2.21±0.06[b]	2.69±0.08[a]	**
Phenylalanine (μmol/kg BW/min)	0.815±0.049[b]	1.040±0.049[ab]	1.138±0.064[a]	*
Tyrosine (μmol/kg BW/min)	0.766±0.065	0.923±0.065	1.022±0.086	NS
Phenylalanine kinetics				
Rate (μmol/kg BW/min)				
To hydroxylation	0.074±0.014	0.094±0.014	0.118±0.018	NS
To protein synthesis	0.741±0.039[b]	0.945±0.039[ab]	1.020±0.052[a]	*
Ratio to total flux				
Hydroxylation	0.091±0.009	0.091±0.009	0.102±0.011	NS
Protein synthesis	0.909±0.009	0.909±0.009	0.898±0.011	NS
WBPS (mg/kg BW/min)	3.68±0.19[b]	4.69±0.19[ab]	5.06±0.25[a]	*

[1] Values represent least squares means±SE of two or three animals.

[2] 1.0×M: energy intake for maintenance. 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than crude protein.

[a, b, c] Different superscripts within a row are significantly different at $p<0.05$.

NS: Not significant, # $p<0.10$, * $p<0.05$, ** $p<0.01$.

lowered ($p<0.01$) ruminal ammonia N concentration, but elevated ($p<0.01$) total VFA concentration. Ruminal acetate and propionate concentrations also increased ($p<0.05$) in response to increasing NPEI, but ruminal isobutyrate concentration decreased ($p<0.05$). Significant effects of NPEI were not found ($p\geq0.10$) on other ruminal VFA concentrations.

During the 2 h period from 5 to 7 h after feeding, plasma glucose concentration tended to be lower ($p<0.10$) for 1.5×M than for 1.0×M and 2.0×M (Table 4). Non-protein energy intake had no significant effects on plasma lactate or FFA concentrations ($p\geq0.10$). Increasing NPEI reduced ($p<0.01$) plasma urea N concentration, but tended to raise ($p<0.10$) plasma total amino N concentration. Plasma phenylalanine concentration tended to increase ($p<0.10$) and plasma tyrosine concentration increased ($p<0.05$) as NPEI increased. Plasma insulin concentration tended to be higher ($p<0.10$) for 2.0×M than 1.0×M and 1.5×M.

During the 2 h period from 5 to 7 h after feeding, plasma enrichments of [$^{13}C_6$]glucose (mean coefficient variation (CV) 6.2%), [$^2H_5$]phenylalanine (mean CV 5.1%), [$^2H_2$]tyrosine (mean CV 8.3%) and [$^2H_4$]tyrosine (mean CV 10.4%) were relatively stable and had no significant time trends from a linear regression analysis in any treatment ($p\geq0.10$, data not shown), showing that metabolism of glucose and these amino acids were in quasi-steady state in this period. Increasing NPEI elevated whole body ILR of glucose ($p<0.01$, Table 4) and phenylalanine ($p<0.05$). However, an increase in tyrosine ILR in response to increasing NPEI was not significant ($p\geq0.10$). The rate of utilization for protein synthesis of phenylalanine increased ($p<0.05$) with increasing NPEI, although that of hydroxylation of phenylalanine did not show a significant increase ($p\geq0.10$). The ratio of the rates to phenylalanine ILR was similar ($p\geq0.10$) for any dietary treatment. The WBPS was elevated ($p<0.05$) with increasing NPEI.

DISCUSSION

Careful interpretation is needed for the present results because of the small number of animals used. Because nutrient absorption is active during 5 to 7 h after feeding in ruminants fed twice daily (Whitt et al., 1996), an increase in WBPS with increasing NPEI in the present study suggests that increasing NPEI may increase WBPS at the absorptive state in adult goats fed twice daily on diets consisting of conventional feeds, and holding a constant ratio of

roughage to concentrate. The agreement between the present results and those of Fujita et al. (2006a), who have shown that increasing energy intake with supplemented starch enhanced WBPS at the absorptive state in goats fed twice daily, confirms the stimulative effect of NPEI on WBPS at the absorptive state in ruminants. In contrast, Nissen and Ostaszeuski (1985) have reported that WBPS decreased with increasing energy intake, by starch supplementation from 1.0 to 1.8 times maintenance requirement at the post-absorptive state (beyond 17 h after feeding) in sheep fed twice daily. Thus, in view of the present and previous results, although the animal species are different, we may assume that in ruminants WBPS increases in response to increasing NPEI at the absorptive state, but decreases at the post-absorptive state. The feeding-related response of WBPS to increasing NPEI has been described by Motil et al. (1981) in humans, who showed that WBPS increased slightly with increasing carbohydrate and fat intake corresponding to 25% of energy for maintenance requirement at a fed state, but remained unchanged at a post-absorptive state.

It appears that the increased WBPS may be largely attributed to an increase in amino acid absorption, as suggested from an increase in plasma tyrosine concentration, trends toward increases in plasma concentrations of total amino N and phenylalanine and an elevated phenylalanine ILR. Increases in concentrations of ruminal total VFA, acetate and propionate and plasma insulin suggest that the release of amino acids from whole body protein breakdown may be suppressed, rather than stimulated, with increasing NPEI, because increases in energy supply (Eskeland et al., 1974; Asplund et al., 1985) and insulin (Tesseraud et al., 1993) have been shown to decrease whole body protein breakdown. The results are also similar to those of Fujita et al. (2006a). The positive response to increasing NPEI in WBPS accompanied by increasing amino acid absorption is probably characteristic of ruminants, because studies in adult humans (Motil et al., 1981) and growing pigs (Reeds et al., 1981) have shown that unchanged flux-this suggests unchanged amino acid absorption-and decreased oxidation of leucine as a tracer produced increased WBPS with increasing carbohydrate or fat intake.

Increased amino acid absorption with increasing NPEI may be also suggested by decreased ruminal ammonia concentration, because decreased ruminal ammonia N concentration with increasing NPEI may result from increases in microbial protein synthesis in the rumen (Merchen et al., 1986; Piwanka et al., 1994) and dietary protein entering the small intestine due to a decrease in ruminal OM digestion (Galyean and Owens, 1991). Furthermore, decreased ruminal ammonia N concentration may result in a decrease in ammonia absorption from the rumen and an increase in urea transfer from blood into the rumen, since ammonia absorption is positively (Nolan, 1993) and urea transfer is negatively (Kennedy and Milligan, 1980) related to ruminal ammonia N concentration. The decrease in ammonia absorption and the increase in the urea transfer may decrease plasma urea N concentration and thereby allow a decrease in urine N and urea N excretion. In consequence of these changes, the form of absorbed N may be shifted from ammonia to amino acid; this may be responsible for improvement in the efficiency of digestible N utilization with increasing NPEI.

Elevated glucose ILR with increasing NPEI agrees with previous results that have shown a positive relationship between glucose turnover and energy intake (Schmidt and Keith, 1983). This elevation may be associated with enhancement in insulin sensitivity (Fujita et al., 2006b). Simultaneous enhancements in WBPS and glucose ILR with increasing NPEI is also in agreement with Fujita et al. (2006a) and thus raise the expectation that increased glucose availability may enhance whole body protein kinetics in ruminants, especially when amino acid availability is high, as described by Fujita et al. (2006a). Although in humans the addition of glucose to an amino acid mixture administered orally augmented a positive effect of amino acid mixture alone on WBPS during fasting (Sim et al., 1979) and on net muscle protein anabolism after resistance exercise (Miller et al., 2003) and at rest (Volpi et al., 2000), only indirect evidence has shown the same expectation in ruminants. Matras and Preston (1989) showed that an intravenous glucose infusion improved N retention without changing plasma concentrations of urea N and insulin in growing wether lambs fed a high-protein diet. Obitsu et al. (2000) indicated increased alanine flux with decreased plasma alanine concentration and a trend toward decrease in urea N from the amino acid in fed sheep with glucose infused into the abomasum. These previous results suggest the stimulation of WBPS with glucose, rather than suppression of whole body protein breakdown. This may be also partly supported by Wester et al. (2004), who have reported that in fasted lambs the fractional rate of protein synthesis in skeletal muscle was numerically increased by an average of 40%, by 6 h intravenous glucose infusion at a rate doubling the glucose entry rate and elevating plasma insulin concentration.

In conclusion, increasing NPEI may enhance WBPS during the absorptive state, and improve the efficiency of digestible N retention in adult male goats fed twice daily. These responses are possibly associated with decreased ammonia and increased amino acid absorption, which may produce a shift in the form of absorbed N from ammonia to amino acid. Simultaneous increases in glucose ILR and WBPS with increasing NPEI raise the expectation of the combined stimulative effect of increasing availabilities of amino acid and glucose on WBPS.

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