WHOLE-BODY PROTEIN TURNOVER IN GOATS ENHANCED BY SUPPLEMENTING A DIET WITH RUMEN PROTECTED METHIONINE

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Summary

Three experiments were conducted with female Japanese Saanen goats to investigate the effects of rumen protected methionine (RPMet) on N utilization and whole-body protein turnover. Whole-body leucine flux from which whole-body protein turnover rates were derived was measured by primed- continuous infusion of L-[^{15}N] leucine in combination with gas chromatography-mass spectrometry. Throughout the experiments RPMet was added to a diet to supply 1.5 g DL-methionine per goat per day. Irrespective of the major N sources (i.e., protein or urea) in the diet, both N deposition and whole-body protein synthesis were increased ($p < 0.05$), and urinary N excretion was decreased ($p < 0.05$) by supplementing with RPMet, but not by supplementing with methionine. It was concluded, therefore, that under the present experimental conditions, the RPMet supplement was efficiently bypassed to result in enhanced body protein synthesis of the goat.
(**Key Words**: Rumen Protected Methionine, Goat, Whole-Body, Protein Turnover)

Introduction

It has been shown that in ruminants methionine may frequently be the first limiting amino acid in diets. This is because methionine is the first-limiting amino acid of rumen bacterial proteins (Storm and Orskov, 1984), the major protein source for ruminants supplying 60 to 85% of the total protein absorbed (Buttery and Foulds, 1988). The methionine deficiency may be alleviated by postruminal infusion of the amino acid (Titgemeyer and Merchen, 1990) or by supplementation with rumen protected methionine (RPMet), which is known to be resistant to degradation in the rumen (Kaufmann and Lupping, 1982).

The beneficial effect of RPMet on lactation and body protein deposition was demonstrated in the literature (Kaufmann and Lupping, 1982 ; Illg et al., 1987; Rogers et al., 1987). Richardson et al. (1976), and Oke et al. (1986) also found improvements in N balance and performance by supplementing with RPMet in the presence or absence of rumen protected lysine.

By extensive literature search, Kaufmann and Lupping (1982) argued that the effect of methionine is not only to balance the amino acid profile so that more amino acids can be available for body and milk protein synthesis but also possibly to promote detoxification of excess ammonia in the liver or to modify metabolism to bring about changes in hormonal status. Although the primary reason for the effect of RPMet is due probably to its effect on balancing amino acid profiles available for body protein synthesis, there is little concrete evidence to support that body protein synthesis of ruminants is actually enhanced *in vivo* by RPMet supplemenation.

Recent development of methodology for measuring body protein turnover *in vivo* with stable isotopes in combination with gas chromatograph-mass spectrometry prompted one to study the N metabolism in ruminants (Krishnamurti and Janssens, 1988; Muramatsu et al., 1988), thus allowing more detailed investigation of impacts of nutrients on body protein kinetics. As pointed out by Harris and Lobley (1991), amino acid metabolism of peripheral tissues in ruminants may not be quite different from that in non-ruminant mammalian species. This might be the case for whole-body protein kinetics, too (Muramatsu,

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1990). It follows, therefore, that if there is a deficiency of any amino acids, supplementing a diet with these amino acids in an available form to the host ruminants should lead to enhanced body protein synthesis in this animal species as demonstrated in single-stomached animals such as rats (Laurent et al., 1984), pigs (Salter et al., 1990), humans (Meredith et al., 1982), and chickens (Muramatsu et al., 1986; Hiramoto et al., 1990; Tesseraud et al., 1992).

Preliminary results suggested that whole-body protein synthesis in goats was increased by supplementing a diet with RPMet (Muramatsu et al., 1989). The present study was conducted to investigate the effect of RPMet on whole-body protein turnover as well as N metabolism in goats.

Materials and Methods

Animals and diets

Three experiments were conducted using female Japanese Saanen goats weighing 25 to 35 kg at 14 to 18 months of age. Animals were cared for under Guidelines for Animal Experimentation, laid down by the Committee of Experimental Animal Care, Nagoya University, Japan. During a 7-day adaptation period, they were given a basal diet as shown in table 1. According to National Research Council (1981), recommended requirements for DCP and DE intake for a 30 kg goat gaining weight at 50 g/day were 45 g/day and 2.03 Mcal/day, respectively. The present intake levels of DCP and DE met these requirements. Extra mineral was provided with a mineral block (Koen, Nihon Zenyaku Co., Fukushima, Japan), which contained (per kilogram): NaCl, 995.5 g; $ZnSO_4$, 1,235 mg; $MnCl_2$, 1,146 mg; Fe_2O_3, 715 mg; $CuSO_4$, 377 mg; KI, 65.5 mg; and $CoSO_4$, 6.6 mg. The goats were then transferred to metabolism cages placed in a clean, air-conditioned room in which ambient temperature was kept at 21 ± 2°C throughout the experimental period. They were given the same diet with or without an amino acid supplement, either 1.5 g of DL-methionine or 5 g of Lactet® (Nihon Soda Co. Ltd., Tokyo, Japan) containing 30% DL-methionine, daily at 1,000 hours for a given period. The RPMet supplement was made by coating methionine with chitosan and fat, attaining protection rate from rumen degradation at about 65% according to the manufacturer. The supplementary level of RPMet at 5 g, i.e. equivalent to 1.5 g DL-methionine per day, was chosen to give unequivocal beneficial effects of RPMet according to our previous results (Muramatsu

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	Expt. 1 and 2		Expt. 3	
	(g/kg)	(g/day)**	(g/kg)	(g/day)**
Dehydrated alfalfa hay cube	381	308	381	308
Wheat bran	619	500	–	–
Semi-purified diet*	–	–	619	500
Calculated :				
DM (g/kg)	879		890	
(g/day)**	710		719	
DCP (g/kg)	106		85	
(g/day)**	86		68	
DE (Mcal/kg)	2.90		2.70	
(Mcal/day)**	2.33		2.18	
Methionine (g/kg)	1.20		0.50	
(g/day)**	1.00		0.39	

** For a goat weighing 30 kg. The values were changed proportionally to metabolic body sizes, $kg^{0.75}$.

* Contained (g/kg): maize starch, 605; glucose, 200; cane molasses, 100; sodium chloride, 20; calcium diphosphate, 20; mineral mixture, 20; and urea, 35. The mineral mixture supplied (g/kg):$(MgCO_3)_4 Mg(OH)_2 5H_2O$, 175.0; K_2CO_3, 767.7; $MnCO_3$, 13.0; Fe citrate $3H_2$, 32.3; $ZnCO_3$, 0.60; $CoCl_2$, 1.00; Na_2MoO_4, 0.10; KI, 0.30; and $CuCl_2$ $2KCl$ $2H_2O$, 10.0.

et al., 1991). Any food left uneaten within the day was weighed to give correction for N intake. Water was provided daily at 2 to 3 L per goat.

Experimental procedures

In Expt 1, four goats were used, and fed on the basal diet for 14 days, followed by feeding the same diet supplemented with RPMet at 5.0 g (supplying 1.5 g DL-methionine) per day for the subsequent 14 days. The goats were then fed on the same basal diet without the RPMet supplement for further 14 days. The last three days of each feeding period were used for collecting faeces and urine to allow N balance measurements.

In both Expt 2 and 3, six goats were used and fed on the basal diet for 7 days, the same diet with DL-methionine at 1.5 g per day for 7 days, and the same diet with RPMet at 5.0 g per day for 7 days. Between the supplementation period, 7-day adaptation periods were allocated so that any residual effects of previous dietary treatments could be avoided. During the last three days of each week of the experimental period, faeces and urine were collected to allow N balance measurements. On the last day of each experimental period (day 7), primed-continuous infusion of L-[^{15}N] leucine (94.6 atom % excess), starting at 1,100 hrs, was done from the left jugular vein at an infusing dose of 0.115-0.135 mg/kg body weight per hr for 6 hrs. The priming dose was equivalent to the 2-hr infusion rate. The priming was done to reach quickly a plateau enrichment of free L-[^{15}N] leucine in plasma (Muramatsu et al., 1988). Blood was sampled from the right jugular vein at hourly intervals during the 6-hr infusion period to ensure that the isotopic equilibrium of free L-[^{15}N] leucine in plasma was attained at the end. If plateau enrichment was not reached in any goats at the end of the infusion, the data were excluded.

Chemical analyses

The N content in faeces, urine and the diet was determined by a Kjeldahl method (AOAC, 1990).

Blood samples were centrifuged at 1,800 g for 10 min, to separate the plasma fractions, which were subsequently deproteinized with 5 volumes of 1% (w/v) picric acid. The deproteinized samples were stored at −20℃ until analysis of the isotopic abundance of [^{15}N] leucine.

The picric acid in the deproteinzed samples was removed by passing through a column of the Cl form of Dowex 2-X8, and the effluent was dried under reduced pressure at 50℃ Free amino acids were derivatized as described by Muramatsu et al. (1987a,b). At first methanol esters of amino acids were formed and converted to butanol esters in order to increase the recovery. An appropriate amount of dried sample dissolved in about 1 ml of de-ionized water was transferred to a reaction vial, and evaporated to dryness at 80℃ in a current of N_2 gas. About 0.2 ml of dichloromethane was then added to the vial, and the sample was again evaporated to dryness at 80° under N_2 gas. This procedure was repeated three times, followed by the addition of 2 ml of absolute methanol to the sample. The HCl gas, which was generated by mixing concentrated HCl with concentrated sulfuric acid, was bubbled through the solution until the vial became hot. After standing for more than 20 min at room temperature, it was evaporated to dryness at 80℃ in a current of N_2 gas. The procedure of dichloromethane addition and evaporation was repeated three times. Subsequently, 2 ml of absolute butanol was added and the HCl gas was bubbled through the solution, which was then maintained at 100℃ for 60 min. After the same evaporation procedure, the sample was finally mixed with 2 ml of dichloromethane/trifluoroacetate (1:1, v/v), and maintained at 100℃ for more than 20 min.

The isotope abundance in n-trifluoroacetyl-butyl-esters thus obtained was analyzed with a computer-controlled selected-ion monitoring gas chromatograph-mass spectrometer (QP-1000, Shimadzu Co. Ltd., Kyoto, Japan). Analytical conditions for separating amino acids were essentially the same as those described by Gehrke et al. (1968), and parameters measuring free [^{15}N] leucine enrichment with the GCMS are given in table 2.

Calculation

Estimation of whole-body leucine flux Q was done as described by Conway et al. (1980) as follows: leucine flux (mg/6 hrs) = (infusate enrichment/plateau enrichment in plasma-1) × ID × F, where ID and F stand for total infusion dose of [^{15}N] leucine, and a factor, 0.93, for correcting

TABLE 2. ANALYTICAL CONDITIONS FOR MEASURING L-[^{15}N] LEUCINE ENRICHMENT BY USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Apparatus	Shimadzu QP-1000**
Column	0.5% Ethylene glycol adipate on chromosorb W+ packed in a glass column of 2.6 mm in diameter and 2,100 mm long
Column temperature	170℃
Separator temperature	250℃
Ion source temperature	250℃
Ionizing voltage	70 eV
Mass numbers of fragment ion	183/182

** Shimadzu Co. Ltd., Kyoto, Japan
+AW 80-100 mesh, Wako Pure Chemical Co. Ltd., Osaka, Japan.

possible overestimation of protein kinetics when [^{15}N] amino acids were used as tracer (Helland et al., 1988).

The calculated leucine flux was converted to protein flux by assuming that leucine accounted for proportionately 0.0684 of body protein in goats (Smith, 1980). The protein flux (Q, grams of protein/day) can be expressed by the following equation : $Q = S + O = I + D$, where S and O stand for protein synthesis and urinary N excretion as excretory end products, and I and D are originally defined by Reeds and Lobley (1980) as protein intake and protein degradation, respectively. Instead of protein intake, however, in the present study, apparently absorbed protein, i.e. product of crude protein intake and digestibility, was used for I as was done previously (Muramatsu et al., 1988). With known rates of Q and I, protein degradation was calculated. Protein synthesis was estimated by subtracting urinary N excretion from the flux on a basis of grams of protein/day. In the above equation, a two-pool model consisting of free amino acid and protein pools in whole body of the goat was assumed. In addition, a time scale for the flux estimation being 6 hours, and for the N balance study being 3 days was different so that the parameters of whole body protein kinetics were assumed to be consistent during the N collection period.

Statistical analysis

A randomized complete block analysis was carried out for all the experiments taking a goat as a block by using the GLM procedure (Statistical Analysis System, 1985). Although the effects of diets and time were confounded in all three experiments, it was shown from the first experiment that the effect of dietary treatment would be primarily, if not entirely, responsible for the changes observed. Therefore, any changes in measurements were regarded as diet effects. When the number of replicates was not equal due to excluded values, least square means were used instead of arithmetic means to assess dietary treatment effects. The significance of differences between treatment means was assessed by Duncan's multiple range test.

Results

The values for urinary N excretion and N balance of goats fed on the basal diet supplemented with or without RPMet are shown in figure 1. A significant decrease in urinary N excretion and increase in N balance were observed when the diet was supplemented with RPMet, and these values returned to the initial levels when RPMet supplement was removed.

Changes in free [^{15}N] leucine enrichment in plasma of goats fed the wheat bran-hay cube diet are given in figure 2. It was indicated that three or four goats out of six reached reasonably well plateau enrichment values for free [^{15}N] leucine in plasma at the end of the infusion.

Effects on N utilization and whole-body protein turnover of supplementing the wheat bran hay cube diet with DL-methionine or RPMet are given in table 3. Faecal N excretion and hence apparent N digestibility were not signific-

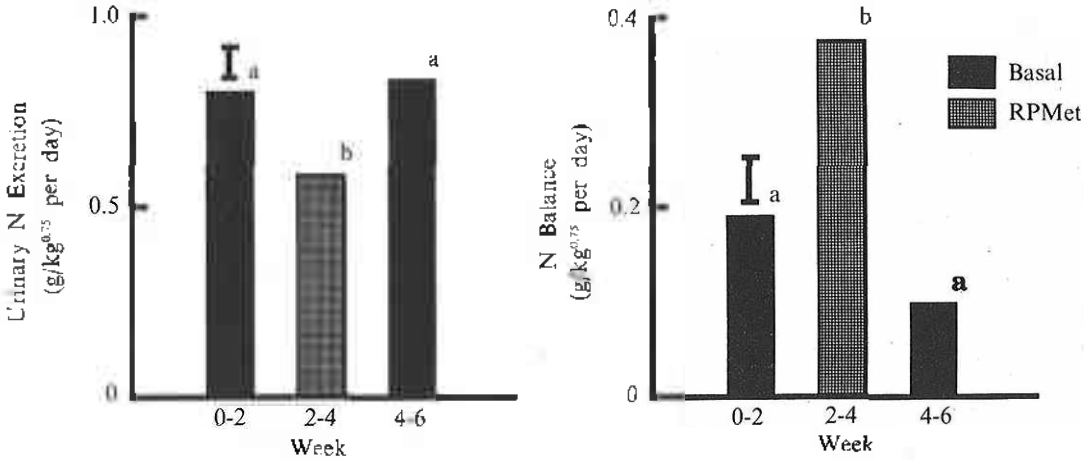

Figure 1. Effects of presence or absence of rumen protected methionine (RPMet) on urinary N excretion and N balance in goats fed on a diet based on wheat bran and hay cube (Expt 1). The RPMet (Lactet®, Nihon Soda Co. Ltd., Tokyo, Japan) containing DL-methionine at 30% was supplemented at a daily dose of 5 g to supply 1.5 g DL methionine per goat. Each bar represents mean of 4 goats, and a small vertical bar, pooled SEM. [a,b] Means lacking a common superscript letter are significantly different at $p < 0.05$.

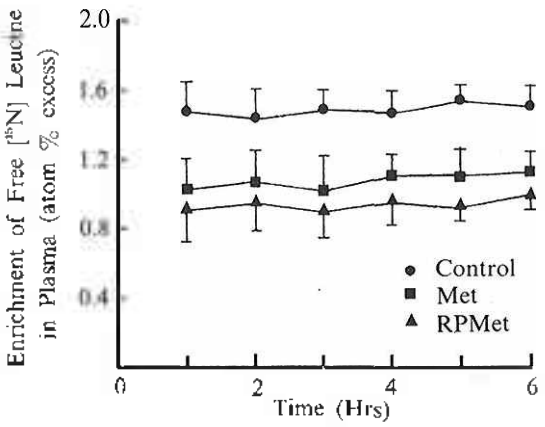

Figure 2. Effects of supplementing with methionine (Met) or rumen protected methionine (RPMet) on enrichment of free [^{15}N] leucine in plasma during primed-continuous infusion of [^{15}N] leucine into a jugular vein of goats (Expt 2). The goats were fed a diet based on wheat bran and hay cube. DL-Methionine and the RPMet (Lactet® containing DL-methionine at 30%, Nihon Soda Co. Ltd., Tokyo, Japan) were fed daily at amount of 1.5 and 5.0 g (1.5 g as DL-methionine) per goat, respectively. Each point and vertical bar represent mean and SEM of 3 (control and Met) or 4 (RPMet) goats, respectively.

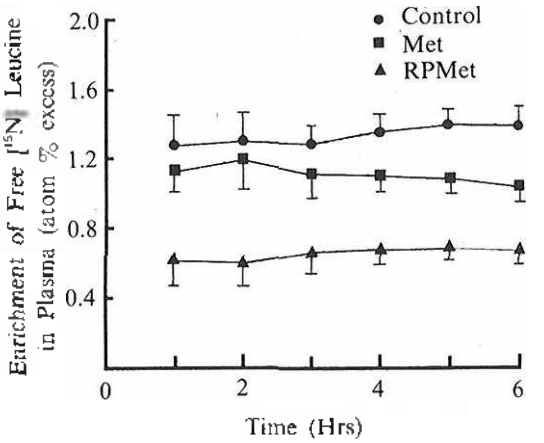

Figure 3. Effects of supplementing with methionine (Met) or rumen protected methionine (RPMet) on enrichment of free [^{15}N] leucine in plasma during primed-continuous infusion of [^{15}N] leucine into a jugular vein of goats (Expt 3). The goats were fed a diet based on urea and hay cube. DL-Methionine and the RPMet (Lactet® containing DL-methionine at 30%, Nihon Soda Co. Ltd., Tokyo, Japan) were fed daily at amounts of 1.5 and 5.0 g (1.5 g as DL-methionine) per goat, respectively. Each point and vertical bar represents mean and SEM of 3 goats, respectively.

antly affected by these supplements. RPMet supplementation decreased urinary N excretion and increased N retained ($p < 0.05$) resulting in improved N utilization ($p < 0.05$) compared to goats fed on the control or DL-methionine supplemented diets. N retention did not differ ($p > 0.05$) between control and DL-methionine supplemented diets. The number of replicates for protein kinetic measurements was reduced as indicated in the table 3 due to the failure of attaining isotopic equilibrium of [^{15}N] leucine. Whole-body protein synthesis was increased ($p < 0.05$) in goats given the RPMet supplement, but not by the DL-methionine supplement when compared with those in goats without supplements. Whole-body protein degradation and leucine flux showed a similar, though not significant, trend towards whole-body protein synthesis.

Figure 3 represents changes in free [^{15}N] leucine enrichment in plasma of goats fed the urea-hay cube diet. As in Expt 2, three goats out of six reached isotopic equilibrium for free [^{15}N] leucine in plasma at the end of the infusion.

In table 4, the values for N utilization and whole-body protein turnover rates are shown when the N source of the diet was mainly from urea in the semi purified diet. Generally, similar results to those with the wheat bran-hay cube diet were found. Namely, there were no significant differences in faecal N excretion and apparent N digestibility between the dietary treatments. In contrast, urinary N excretion was reduced ($p < 0.05$), and both N retained and N utilization were increased by RPMet supplementation ($p < 0.05$) but not by DL-methionine supplementation. The number of replicates for protein kinetic measurements was reduced for the same reason as in Expt 2. The RPMet supplementation increased leucine flux and whole-body protein synthesis rate compared with those in unsupplemented controls ($p < 0.05$). On this occasion, whole-body protein degradation rate was also increased by supplementing with RPMet ($p < 0.05$). None of these protein kinetic parameters was significantly changed by supplementing with DL-methionine.

TABLE 3. EFFECT OF FEEDING METHIONINE OR RUMEN PROTECTED METHIONINE (RPMET)** ON NITROGEN UTILIZATION AND WHOLE-BODY PROTEIN TURNOVER IN GOATS FED A DIET BASED ON WHEAT BRAN AND HAY CUBE (EXPT. 2)

Item	Supplement*			SEM (10 df)
	None (control)	Met	RPMet	
No. of replicates	6	6	6	—
Body wt. (kg)	32.0	32.6	31.5	0.4
N intake (g/kg$^{0.75}$/day)	1.34	1.35	1.38	0.02
N excreted (g/kg$^{0.75}$/day)				
In feces	0.32	0.31	0.29	0.01
In urine	0.88^{a}	0.86^{a}	0.67^{b}	0.04
N retained (g/kg$^{0.75}$/day)	0.14^{a}	0.18^{a}	0.42^{b}	0.04
N digestibility	0.77	0.78	0.79	0.01
N utilization*+	0.06^{a}	0.09^{a}	0.27^{b}	0.03
Leucine flux (g/kg$^{0.75}$/day)	0.61	0.77	0.86	0.26
Whole-body protein turnover				
Synthesis (g/kg$^{0.75}$/day)	5.0^{a}	7.6^{ab}	9.9^{b}	1.1
Degradation (g/kg$^{0.75}$/day)	4.5	6.5	7.9	1.4

** Lactel® (Nihon Soda Co. Ltd., Tokyo, Japan) containing DL-methionine at 30%.

* DL-methionine and RPMet were fed daily at amounts of 1.5 and 5.0 g (1.5 g as DL-methionine) per goat, respectively.

*+ N retained/N intake.

The number of replicates was reduced to 3, 3 and 4 for control, Met and RPMet respectively, due to the failure of attaining isotopic equilibrium in plasma at the end of primed-continuous infusion for 6 hrs.

a,b Means within the same row lacking a common superscript letter are significantly different at $p < 0.05$.

TABLE 4. EFFECT OF FEEDING METHIONINE OR RUMEN PROTECTED METHIONINE (RPMET)** ON NITROGEN UTILIZATION AND WHOLE-BODY PROTEIN TURNOVER IN GOATS FED A DIET BASED ON UREA AND HAY CUBE (EXPT. 3)

Item	Supplement+			SEM (7 df)
	None (control)	Met	RPMet	
No. of replicates	5	5	4	–
Body wt. (kg)	29.9	30.3	30.5	2.2
N intake (g/kg$^{0.75}$/day)	1.26	1.28	1.27	0.02
N excreted (g/kg$^{0.75}$/day)				
In feces	0.40	0.42	0.41	0.02
In urine	0.64^{a}	0.60^{a}	0.40^{b}	0.03
N retained (g/kg$^{0.75}$/day)	0.16^{a}	0.20^{a}	0.43^{b}	0.04
N digestibility	0.69	0.68	0.69	2.0
N utilization++	0.13^{a}	0.16^{a}	0.36^{b}	3.4
Leucine flux (g/kg$^{0.75}$/day)	0.76^{a}	0.92^{a}	1.35^{b}	0.06
Whole-body protein turnover				
Synthesis (g/kg$^{0.75}$/day)	8.4^{a}	10.6^{a}	18.2^{b}	1.0
Degradation (g/kg$^{0.75}$/day)	7.3^{a}	9.4^{a}	15.2^{b}	0.8

** Lactet® (Nihon Soda Co. Ltd., Tokyo, Japan) containing DL-methionine at 30%.

+ DL-methionine and RPMet were fed daily at amounts of 1.5 and 5.0 g (1.5 g as DL-methionine) per goat, respectively.

++ N retained/N intake.

The number of replicates was reduced to 3 for all treatments due to the failure of attaining isotopic equilibrium in plasma at the end of primed-continuous infusion for 6 hrs.

^{a,b} Means within the same row lacking a common superscript letter are significantly different at $p < 0.05$

Discussion

It has been found in ruminants that under certain circumstances, RPMet addition to a diet could improve daily weight gain, N deposition, and milk production (Kaufmann and Lupping, 1982; Illg et al., 1987). In the present study, the beneficial effect of RPMet supplementation on N deposition was also demonstrated (figure 1, and tables 3 and 4). In Expt 1, although the effect of RPMet feeding was confounded with that of time, the fact that urinary N excretion and N balance returned to the initial levels by switching the diets from RPMet to basal suggested that observed changes were due primarily to the effects of feeding RPMet *per se*. The present results were in good agreements with previous findings with goats (Muramatsu et al., 1991). Under similar dietary conditions, methionine and lysine were found to be the first- and second-limiting amino acids respectively, in growing goats (Muramatsu et al., 1993). When the same RPMet was supplemented, an apparent rumen-bypass rate of RPMet was estimated to be 60% as calculated by changes in N deposition, giving responses equivalent to four to five-fold amounts of methionine per se (Muramatsu et al., 1991).

To our best knowledge, however, there was little concrete evidence to demonstrate that body protein synthesis *in vivo* of ruminants was actually enhanced by RPMet supplementation except for the preliminary result of Muramatsu et al. (1989). It was clearly indicated in the present study (tables 3 and 4) that irrespective of N sources, increased N deposition by supplementing with RPMet was brought about essentially by enhanced whole-body protein synthesis which was accompanied by increased, though to a lesser extent, whole-body protein degradation. In this respect, the present study may be the first indication of enhanced whole-body protein synthesis after administration of RPMet when the N intake was kept adequate to meet the requirement level as recommended by NRC (1981). The increased whole-body protein synthesis by supplementing with RPMet resulted in decreased amino acid oxidation, another major component of amino acid efflux, which was exemplified by reduced

urinary N excretion (table 3 and 4). The beneficial effects of RPMet seem to be restricted to certain N intake levels because changes in N balance were no longer apparent when goats were fed on a high protein deit based upon soybean meal and hay cube (unpublished results).

The assumptions used in the present study to calculate whole-body protein kinetics were, of course, gross oversimplification. Firstly, identification of the true precursor pool for protein synthesis is of considerable importance for the estimation of whole-body protein turnover. There seems general agreement that the intracellular α-ketoisocaproate could be considered as a direct precursor for leucine oxidation, and therefore its use for estimation could give accurate estimates for protein synthesis. However, Petrides et al. (1991) have suggested that the changes in leucine flux by experimental treatments should be similar whether plasma leucine or plasma α-ketoisocaproate is used as a precursor. Secondly, the status of insulin and substrate availability may seriously affect whole body leucine kinetics, particularly endogenous leucine appearance which is considered as a measure of whole-body protein degradation, as suggested by Tesseraud et al. (1993). Thirdly, leucine kinetics in a fed state is complicated by the presence of 'first pass' effect, i.e. partial extraction of leucine flux by the liver. No adjustment for the above factors was taken into account in the present study. However, in studies with a whole-animal, simplification as employed in the present study cannot be avoided. Problems associated with the two-pool model and methodology were discussed elsewhere in detail (Waterlow et al., 1978).

In addition to common problems concerning protein kinetic measurements in the whole-body, the use of ruminants as experimental animals introduces further error sources. The values for whole-body protein degradation were derived by subtracting absorbed protein (N × 6.25) from the protein flux. In ruminants, however, ammonia absorbed from the portal-drained viscera may be much higher than N absorbed in the form of amino acids (Huntington, 1986). Because of this, the calculation of whole-body protein degradation might have been disturbed to an unknown extent in the present study. If there is a significant amount of urea N recycling within the goat body as in other ruminants (Huntington, 1986), the estimates of whole-body protein synthesis based on the flux and urinary N excretion would also be affected. This might be the case as implied by the differences in overall turnover rates of the last two experiments. The large difference found between the two basal diets cannot simply be attributable to random variances between experiments, but at least to a certain extent to the presence or absence of dietary urea as the major dietary N source. Nevertheless, the present estimates of whole-body protein turnover in the goat may still be good in a relative term to compare treatment effects, provided that uptake and transport of nonprotein N were not seriously affected by supplementing the diet with small amounts of DL-methionine or RPMet.

According to Chalupa (1976), and Cottle and Velle (1989), methionine may be relatively resistant to degradation in the rumen among various amino acids. When goats are given a large amount, such as 5 g or more per day, substantial amounts of methionine could escape from degradation in the rumen, and thereby reach the lower gut (Cottle and Velle, 1989). If this is the case, supplementing with a large amount of intact DL-methionine could improve N utilization and whole-body protein synthesis of goats as expected from the results with the RPMet supplement. Indeed, by increasing the amount of methionine supplementation, a good linear dose response relationship was found between N deposition and dietary DL-methionine supplements up to 9 g per goat per day when the amino acid was given in the intact form (Muramatsu et al., 1991). However, the amount employed in the present study, at 1.5 g per goat per day, was too small to have any significant effects. Assuming the degradability of intact DL-methionine in the rumen at 90%/day (Cottle and Velle, 1989), only 0.15 g of the supplemented DL-methionine could reach the lower gut to be absorbed under the present experimental conditions.

In the literature, there seems to be species differences in responsiveness to RPMet supplementation. The effect was clearer in sheep than in cattle (Kaufmann and Lupping, 1982). Therefore, it may be stated that the responses to RPMet obtained with goats are close to those found in sheep. Investigation into RPMet effects on the species differences remains to be done.

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