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Comparison of the [$^2H_5$]Phenylalanine Model with the [1-^{13}C]Leucine Method to Determine Whole Body Protein Synthesis and Degradation in Sheep Fed at Two Levels

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ABSTRACT : The [$^2H_5$]phenylalanine model was compared with the [1-^{13}C]leucine method to determine whole body protein synthesis (WBPS) and degradation (WBPD) in sheep fed at two levels. The animals were fed either 103 (M-diet) or 151 (H-diet) kcal ME/kg$^{0.75}$/day once daily in a crossover design for 21 days each. The isotope dilutions were simultaneously conducted as a primed-continuous infusion of [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine and [1-^{13}C]leucine on each dietary treatment. The WBPS and WBPD calculated from the [$^2H_5$]phenylalanine model were lower ($p = 0.009$ and $p = 0.003$, respectively) than those calculated from the [1-^{13}C]leucine method. The WBPS tended to be higher ($p = 0.08$) and WBPD was numerically higher ($p = 0.33$) for H-diet than M-diet in the [$^2H_5$]phenylalanine model, whereas the WBPS was numerically higher ($p = 0.37$) for H-diet and WBPS remained similar ($p = 0.79$) between diets in the [1-^{13}C]leucine method. However, the absolute values and the directions of WBPS as well as WBPD from M-diet to H-diet were comparable between the [$^2H_5$]phenylalanine model and [1-^{13}C]leucine method. Moreover, the values vary depending on the use of the respective amino acid contents in the carcass protein when calculating WBPS and WBPD. Therefore, it is concluded that the [$^2H_5$]phenylalanine model could be used as an alternative to the [1-^{13}C]leucine method for the determination of WBPS and WBPD in sheep. (**Key Words** : Isotope Dilution Method, Stable Isotope, Dietary Intake, Protein Synthesis, Protein Degradation, Sheep)

INTRODUCTION

A number of well-established isotope dilution techniques using radioactive and stable isotopes have been used to estimate whole body protein metabolism in humans and animals (Wolfe, 1984; Marchini et al., 1993; Sano et al., 2004). Among the methods, isotope kinetics of [1-^{13}C]leucine is the most widely used method for the assessment of whole body protein synthesis (WBPS) and degradation (WBPD) through the measurement of plasma α-[1-^{13}C]ketoisocaproic acid (α-[1-^{13}C]KIC), the true precursor of intracellular leucine metabolism, enrichment in humans, sheep, and cows (Matthews et al., 1982; Krishnamurti and Janssens, 1988; Lapierre et al., 2002; Sano et al., 2004). The [$^2H_5$]phenylalanine model, as proposed by Clarke and Bier (1982), could also be used for the accurate measurement of WBPS and WBPD quickly and, in addition, it offers the advantage of not requiring the measurement of expired air CO_2 production or $^{13}CO_2$ enrichment, which enables the analyses to be performed using gas chromatography-mass spectrometry (GC/MS) alone.

Although, the [$^2H_5$]phenylalanine model has been used in humans and animals (Pacy et al., 1994; Borel et al., 1997; Clark et al., 1997; Whittaker et al., 1999; Gibson et al., 2002; Bregendahl et al., 2004; Fujita et al., 2006) for the determination of whole body protein metabolism, a few experiments have been reported in sheep (Harris et al., 1992; Connell et al., 1997; Lobley et al., 2003). Harris et al. (1992) used [^{3}H-2.6]phenylalanine and [U-^{14}C]phenylalanine and stated that WBPS increased with increased nutritional level. However, they used previous nitrogen balance data (Harris et al., 1989) to calculate WBPD without measuring nitrogen balance simultaneously. In another study, Connell et al. (1997) used [1-^{13}C]glycine, [1-^{13}C]leucine and [$^2H_5$]phenylalanine and stated that irreversible loss rate (ILR) and whole body protein flux (WBPF) were higher for fed than fasted sheep. However, the authors did not measure the oxidation of leucine to CO_2 via α-KIC and phenylalanine to tyrosine. Furthermore, there is some debate about the application of the [$^2H_5$]phenylalanine

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model which has been elaborated by Fujita et al. (2006). Dietary energy intake also influences whole body protein metabolism in goats (Fujita et al., 2006).

The present experiment was conducted using isotope dilution and nitrogen balance tests simultaneously in sheep fed at two levels of dietary intake to determine whether or not the same WBPS and WBPD could be obtained from the [$^2H_5$]phenylalanine model and the [1-^{13}C]leucine method.

MATERIALS AND METHODS

Animals, diets and management

Four cross-bred (Suffolk×Corriedale) sheep, aged around two years and weighing, initially, 44±1 kg, were used. The animals were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery at least three months before starting the experiment. During the adjustment period the animals were kept in individual pens in an animal barn, and were shorn closely before starting the experiments. The animals were fed mixed hay of orchardgrass and reedcanary grass (1.8 kcal metabolizable energy (ME)/g and 12% crude protein (CP)) and concentrate mixture (2.8 kcal ME/g and 12% CP) in a 1:1 ratio at two different levels, 103 kcal ME/kg$^{0.75}$/day (M-diet) and 151 kcal ME/kg$^{0.75}$/day (H-diet), equivalent to 107 and 157%, respectively, of the ME required for maintenance (National Research Council, 1985), with a crossover design for a period of 21 days on each diet. The sheep were fed at 14:00 h everyday with *ad libitum* water access. On day 15 the sheep were moved to metabolic cages in an environmentally controlled room with an air temperature of 20±1°C. A polyvinyl catheter was placed in the right jugular vein one day before the experiment. Also, a catheter for blood sampling was inserted into the skin loop of the left carotid artery about 2 h before starting the experiment. The catheters were kept flushed with 3.8% (w/v) trisodium citrate sterile solution. The sheep were moved back to individual pens after completing each dietary treatment. They were weighed at the onset, on day 15 and after finishing each dietary treatment. The handling of animals including surgery and blood sampling was carried out according to the guidelines established by the Animal Care Committee of the Iwate University.

Nitrogen balance test

Nitrogen (N) balance was determined for five successive days during the last week of each 3-week treatment period. Urine was collected from each sheep for 24 h in a bucket containing 50 ml of 6N H_2SO_4 and an aliquot was stored at -30°C until analysis. Feces were also collected from each sheep for each 24 h, dried in a forced air oven, ground to 1 mm mesh and stored until analysis as described previously (Pen et al., 2006).

Isotope dilution methods

Two isotope dilution procedures, the [$^2H_5$]phenylalanine model and the [1-^{13}C]leucine method, for the determination of turnover rates of plasma phenylalanine (PheTR), tyrosine (TyrTR) and leucine (LeuTR) and CO_2 production derived from leucine oxidation (LeuOX), were carried out simultaneously on day 21 of each dietary treatment. At 10:00 h 10 ml of 0.9% (w/v) NaCl solution containing 7 μmol/kg$^{0.75}$ of [$^2H_5$]phenylalanine (L-phenyl-d_5-alanine, 98 atom% D excess, Isotec Inc, A Matheson tri-gas Company, USA), 3.5 μmol/kg$^{0.75}$ of [$^2H_2$]tyrosine (L-tyrosine-3, 5-D_2, 98 atom% D excess, Isotec Inc, A Matheson tri-gas Company, USA), 2 μmol/kg$^{0.75}$ of [$^2H_4$]tyrosine (L-4-hydroxyphenyl-2, 3, 5, 6-D_4-alanine, 98 atom% D excess, Isotec Inc, A Matheson, USA Co., USA), 10 μmol/kg$^{0.75}$ of [1-^{13}C]leucine (L-leucine, 99 atom%, Cambridge Isotope Laboratories, Inc, USA), and 3.5 μmol/kg$^{0.75}$ of $NaH^{13}CO_3$ ($NaHCO_3$-^{13}C, 99 atom%, Cambridge Isotope Laboratories, Inc, USA) was injected through the jugular catheter as a priming dose (Wolfe, 1984). Then [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine and [1-^{13}C]leucine were continuously infused by a multichannel peristaltic pump (AC-2120, Atto Co, Ltd., Japan) at a rate of 7.0, 3.5 and 10.0 μmol/kg$^{0.75}$/h, respectively (Sano et al., 2004; Fujita et al., 2006), through the same catheter for 5 h. Blood samples (5 ml each) were taken from the carotid artery catheter immediately before and half-hourly between 3 and 5 h of the infusion of [$^2H_5$]phenylalanine, [$^2H_2$]tyrosine and [1-^{13}C]leucine. The blood samples were transferred into sodium heparinated centrifuge tubes, chilled with crushed ice and then centrifuged at 10,000×g for 10 min at 2°C (RS-18IV, Tomy, Japan) and the plasma stored at -30°C until further analysis.

Open circuit calorimetry

The production of CO_2 and metabolic heat production (HP) were determined using open circuit calorimetry (Metabolic monitor, Coast Electronics, Kent, UK). An aliquot of exhaled CO_2 was trapped in 4 ml of 1 N NaOH every 30 min immediately before and between 3 and 5 h after the initiation of [1-^{13}C]leucine isotope infusion for the determination of $^{13}CO_2$ enrichment.

Chemical analyses

Nitrogen in diets, feces and urine was analyzed with the ammonia N measurement by a colorimetric method (Weatherburn, 1967) after Kjeldahl digestion. Plasma amino acids and α-keto acids were separated and converted to *N*-methyl-*N*-t-butyl-dimethylsilyltrifluoroacetamide (MTBSTFA) derivatives, according to the procedures of Rocchiccioli et al. (1981) and Calder and Smith (1988), for the

Table 1. Effect of dietary intake on nitrogen intake, balance and digestibility and metabolic heat production (HP) in sheep

	Treatment*		SEM[1]	p- value
	M-diet	H-diet		
Body weight (kg)	44	46	2	0.43
Body weight gain (kg/d)	-0.23	0.18	0.07	0.06
N intake (g/kg$^{0.75}$/d)	0.84	1.25	0.08	0.0008
N in feces (g/kg$^{0.75}$/d)	0.16	0.34	0.04	0.01
N absorbance (g/kg$^{0.75}$/d)	0.69	0.90	0.05	0.05
N in urine (g/kg$^{0.75}$/d)	0.27	0.30	0.02	0.13
N balance (g/kg$^{0.75}$/d)	0.42	0.60	0.04	0.08
N digestibility (%)	81	72	3	0.07
HP (kcal/kg BW$^{0.75}$/h)	2.8	3.4	0.3	0.40

* M-diet: 103 kcal ME/kg$^{0.75}$/d, H-diet: 151 kcal ME/kg$^{0.75}$/d.
[1] Standard errors of the means, N: nitrogen.

determination of the isotopic enrichments of plasma [$^2H_5$]phenylalanine, [$^2H_5$]tyrosine, [$^2H_2$]tyrosine, [1-^{13}C]leucine and α-[1-^{13}C]KIC as described previously (Sano et al., 2004; Fujita et al., 2007). These enrichments (mol% excess) were measured by an electron impact ionization-selected ion monitoring method using a GC/MS (QP-2010, Shimadzu, Kyoto, Japan). The isotopic abundance of $^{13}CO_2$ was determined using gas chromatography combustion-mass spectrometry and isotopic-ratio-mass spectrometry (DELTA^{plus}, Thermo Electron Corp., Waltham, MA, USA). Plasma amino acid concentrations were determined using an automated amino acid analyzer (JLC-500/V, JEOL, Tokyo, Japan).

Calculations

Mean values with standard errors of the means (SEM) were used. The turnover rates (TR) of phenylalanine (PheTR), tyrosine (TyrTR) and leucine (LeuTR) and oxidation (OX) of the phenylalanine (PheOX) and leucine (LeuOX). WBPS and WBPD were calculated using the equations described by Krishnamurti and Janssens (1988) and Harris et al. (1992).

$$TR = I \times (1/E-1)$$

Where, I is the infusion rate of each isotope and E is the respective plasma isotope enrichment during steady state.

$$LeuOX = E_{CO2}/E_{Leu}/0.81 \times V_{CO2}$$

Where, E_{CO2} is the isotope enrichment of exhaled $^{13}CO_2$ and V_{CO2} is the CO_2 production rate. The recovery fraction of $^{13}CO_2$ to [^{13}C]bicarbonate was estimated to be 0.81 as used (Sano et al., 2004).

The WBPS calculated from the [1-^{13}C]leucine method ($WBPS_{leu}$) was derived as follows:

$$WBPS_{leu} = (LeuTR-LeuOX) / \text{leucine concentration in carcass protein}$$

The rate of phenylalanine hydroxylation (the rate of phenylalanine conversion to tyrosine, PheOX) was calculated as described by Thompson et al. (1989).

$$PheOX = TyrTR \times (E_{tyr}/E_{phe}) \times (PheTR/(I_{phe}+PheTR))$$

Where, E_{phe} and E_{tyr} are the respective plasma enrichments of [$^2H_5$]phenylalanine and [$^2H_4$]tyrosine, and I_{phe} is the infusion rate of [$^2H_5$]phenylalanine. The WBPS calculated from the [$^2H_5$]phenylalanine model ($WBPS_{phe}$) was derived as follows:

$$WBPS_{phe} = (PheTR-PheOX) / \text{phenylalanine concentration in carcass protein}$$

Whole body protein synthesis was calculated by assuming that the phenylalanine and leucine contents of the body protein are 0.035 and 0.066 g/g CP, respectively (Harris et al., 1992).

Whole body protein synthesis and WBPD were also calculated from the relationship among WBPF, N absorption and urinary N excretion according to the equations described by Schroeder et al. (2006) as follows:

$$WBPS = WBPF-(\text{urinary N excretion} \times 6.25)$$

$$WBPD = WBPF-(\text{N absorbed} \times 6.25)$$

The WBPF was obtained by dividing the PheTR and LeuTR by 0.035 and 0.066, respectively (Harris et al., 1992).

Metabolic heat production was determined according to the Brouwer's (1965) equation with slight modification by Young et al. (1975).

$$HP\ (kcal) = 3.866 \times O_2 \text{ consumption (l)} + 1.2 \times CO_2 \text{ production (l)}$$

Statistical analysis

All data were analyzed with the MIXED procedure of

Table 2. Effect of dietary intake on plasma amino acid concentrations in sheep

μmol/L	Treatment*		SEM[1]	p-value
	M-diet	H-diet		
No. of sheep	4	4		
Arg	66	84	8	0.38
His	37	34	4	0.23
Ile	40	46	4	0.21
Leu	61	67	6	0.25
Lys	58	67	8	0.41
Met	7	8	1	0.41
Phe	25	28	2	0.18
Thr	101	119	17	0.20
Val	83	105	8	0.22
Ala	120	116	12	0.94
Glu	39	35	3	0.43
Gly	462	353	75	0.36
Pro	51	57	5	0.14
Ser	109	88	14	0.13
Asn	24	29	3	0.50
Gln	177	195	20	0.47
Tyr	33	41	4	0.88
Trp	18	20	2	0.26

* M-diet: 103 kcal ME/kg$^{0.75}$/d, H-diet: 151 kcal ME/kg$^{0.75}$/d.
[1] Standard errors of the means.

SAS (1996). The crossover design was used to test the effects of period and method. The crossover design was also used to test the effects of period and diet. The random effect was sheep. Results were considered significant at the $p<0.05$ level. and a tendency was defined as $0.05\leq p<0.10$.

RESULTS

Nitrogen balance, free plasma amino acid and HP

The body weight gain during the experimental period tended to be higher ($p = 0.06$) for H-diet than M-diet (Table 1). Nitrogen intake and N excretion through feces were significantly higher ($p = 0.0008$ and $p = 0.01$, respectively) for H-diet than M-diet, whereas N excretion through urine did not differ significantly ($p = 0.13$) between dietary treatments. Nitrogen balance tended to be higher ($p = 0.08$). but N digestibility tended to be lower ($p = 0.07$) for H-diet than M-diet.

The HP was numerically higher ($p = 0.40$) for H-diet than M-diet. The concentrations of plasma free amino acids remained unchanged between dietary treatments (Table 2).

Protein synthesis and degradation

Plasma concentrations of phenylalanine. tyrosine. leucine and α-KIC and the isotopic enrichments of plasma [$^2H_5$]phenylalanine, [$^2H_4$]tyrosine, [$^3H_2$]tyrosine. [1-^{13}C]leucine and α-[1-^{13}C]KIC were stable during the last 2 h of infusion (data not shown). Enrichment of exhaled $^{13}CO_2$ was also stable during the corresponding period. Turnover rate of

Table 3. Effects of dietary intake on the turnover and oxidation rates of leucine and phenylalanine and whole body protein synthesis (WBPS) and degradation (WBPD) in sheep

	Treatments*		SEM[1]	p-value
	M-diet	H-diet		
No. of sheep	4	4		
Phenylalanine				
Concentration (μmol/L)	32	33	1	0.62
Turnover rate (μmol/kg$^{0.75}$/h)	106	130	6	0.07
Hydroxylation rate (μmol/kg$^{0.75}$/h)	10	14	1	0.17
WBPS (g/kg$^{0.75}$/d)	10.8	13.1	0.6	0.08
WBPD (g/kg$^{0.75}$/d)	8.2	9.4	0.5	0.33
Tyrosine				
Concentration (μmol/L)	39	42	2	0.20
Turnover rate (μmol/kg$^{0.75}$/h)	114	148	11	0.18
Leucine				
Concentration (μmol/L)	89	103	5	0.07
Turnover rate (μmol/kg$^{0.75}$/h)	223[a]	265	11	0.009
Oxidation rate (μmol/kg$^{0.75}$/h)	36[b]	52	7	0.23
WBPS (g/kg$^{0.75}$/d)	8.9	10.3	0.5	0.12
WBPD (g/kg$^{0.75}$/d)	6.3	6.6	0.6	0.69
α-KIC[2]				
Concentration (μmol/l)	13	11	1	0.06
Turnover rate (μmol/kg$^{0.75}$/h)	345	378	11	0.02
Oxidation rate (μmol/kg$^{0.75}$/h)	54	75	10	0.30
WBPS (g/kg$^{0.75}$/d)	13.8	14.7	0.5	0.37
WBPD (g/kg$^{0.75}$/d)	11.2	11.0	0.6	0.79

* M-diet: 103 kcal ME/kg$^{0.75}$/d, H-diet: 151 kcal ME/kg$^{0.75}$/d.
[1] Standard errors of the means. [2] α-Ketoisocaproate.
[a] Significantly lower ($p<0.0001$). [b] Tended to be lower ($p = 0.06$) than that of α-[1-^{13}C]KIC.

Table 4. Effects of dietary intake on whole body protein synthesis (WBPS) and degradation (WBPD) in sheep calculated according to the equation of Schroeder et al. (2006)

	Treatments*		SEM[1]	p-value
	M-diet	H-diet		
No. of sheep	4	4		
Phenylalanine				
WBPS (g/kg$^{0.75}$/d)	10.3	12.8	0.6	0.07
WBPD (g/kg$^{0.75}$/d)	7.7	9.1	0.5	0.25
Leucine				
WBPS (g/kg$^{0.75}$/d)	9.0	10.7	0.4	0.02
WBPD (g/kg$^{0.75}$/d)	6.3	7.0	0.4	0.16
α-KIC[2]				
WBPS (g/kg$^{0.75}$/d)	14.8	16.1	0.4	0.06
WBPD (g/kg$^{0.75}$/d)	12.2	12.4	0.3	0.14

* M-diet: 103 kcal ME/kg$^{0.75}$/d. H-diet: 151 kcal ME/kg$^{0.75}$/d.
[1] Standard errors of the means.
[2] α-Ketoisocaproate.

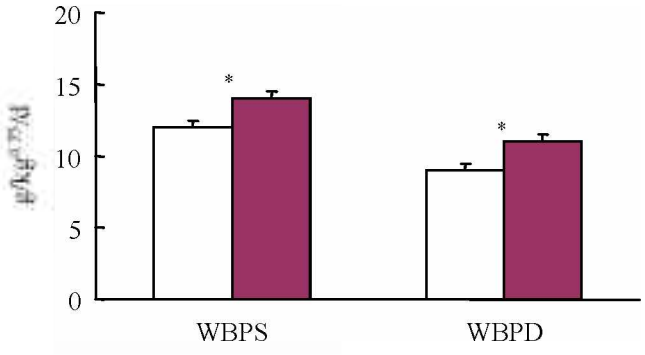

Figure 1. Comparison of whole body protein synthesis (WBPS) and degradation (WBPD) (g/kg BW$^{0.75}$/d, pooled values of both the M- and H-diet) between [$^2H_5$]phenylalanine model (open bars) and [1-^{13}C]leucine method (filled bars). * $p<0.01$.

phenylalanine tended to be higher ($p = 0.07$) for H-diet than M-diet, whereas that of tyrosine was numerically higher ($p = 0.18$) for H-diet than M-diet (Table 3). Plasma phenylalanine hydroxylation rate was also numerically higher ($p = 0.17$) for H-diet than M-diet.

The plasma LeuTR and LeuOX rate calculated from plasma [1-^{13}C]leucine enrichment were lower ($p<0.0001$) and tended to be lower ($p = 0.06$), respectively, than those calculated from plasma enrichment of α-[1-^{13}C]KIC on both dietary treatments. The plasma LeuTR rates calculated from plasma enrichment of [1-^{13}C]leucine and α-[1-^{13}C]KIC were higher ($p = 0.009$ and $p = 0.02$, respectively) for H-diet than M-diet. The LeuOX calculated from plasma enrichment of [1-^{13}C]leucine and α-[1-^{13}C]KIC were numerically higher ($p = 0.23$ and $p = 0.30$, respectively) for H-diet than M-diet.

The WBPS and WBPD calculated by the [$^2H_5$]phenylalanine model tended to be higher ($p = 0.08$) and was numerically higher ($p = 0.33$), respectively, for H-diet than M-diet. However, the WBPS and WBPD calculated from the plasma enrichments of α-[1-^{13}C]KIC remained similar ($p = 0.37$ and $p = 0.79$, respectively) between the dietary treatments, and those calculated from the plasma enrichments of [1-^{13}C]leucine were numerically higher ($p = 0.12$) for H-diet than M-diet and remained similar ($p = 0.69$), respectively, between dietary treatments.

The WBPS and WBPD (pooled values of both the H- and M-diet) calculated from the [$^2H_5$]phenylalanine model were significantly lower ($p = 0.009$ and $p = 0.003$, respectively) than those from the [1-^{13}C]leucine method in which the plasma enrichment of α-[1-^{13}C]KIC was used (Figure 1).

In both [$^2H_5$]phenylalanine model and [1-^{13}C]leucine method, the WBPS tended to be higher ($p = 0.07$ and $p = 0.06$, respectively) and WBPD was numerically higher ($p = 0.25$ and $p = 0.14$, respectively) for H-diet than M-diet (Table 4) when calculated using the equation described by Schroeder et al. (2006).

In the [$^2H_5$]phenylalanine model, the WBPS and WBPD were similar ($p = 0.63$ and 0.52, respectively) between the equations described by Schroeder et al. (2006) and Thompson et al. (1989), whereas in the [1-^{13}C]leucine method the WBPS and WBPD were higher ($p = 0.03$ and 0.01, respectively) for the equations described by Schroeder et al. (2006) than those of Krishnamurti and Janssens (1988).

DISCUSSION

The present experiment was conducted to access the [$^2H_5$]phenylalanine model in sheep, comparing directly with the widely used [1-^{13}C]leucine isotope method, for the determination of WBPS and WBPD *in vivo*. The [$^2H_5$]phenylalanine model is less tedious because there is no need to determine exhaled CO_2 production for the determination of WBPS in ruminants (Fujita et al., 2006). However, Zello et al. (1994) stated that use of the [$^2H_5$]phenylalanine model *in vivo* may not be appropriate where cellular transport mechanisms are involved, because in adult men [$^2H_5$]phenylalanine resulted in significantly higher isotope enrichment in urine than in plasma. Lobley et al. (2003) used a continuous infusion of [1-^{13}C]phenylalanine and [$^2H_4$]tyrosine to determine both the oxidation to CO_2 and the hydroxylation of phenylalanine to tyrosine in wether lambs, and stated that the value of phenylalanine oxidation calculated based on hydroxylation to tyrosine was lower than that calculated from $^{13}CO_2$ production. To correct underestimation resulting from these problems, some researchers in human studies have adopted correction factors derived empirically (Millward et al., 1991; Marchini et al., 1993; Pacy et al., 1994). However, the correction factor proposed for humans could not be applied directly in ruminants. Moreover, Fujita et al. (2006)

used the [$^2H_5$]phenylalanine model to determine WBPS in adult goats without using the correction factor. Therefore, the correction factor was also not used in the present study.

Plasma amino acid turnover and oxidation

The lower plasma LeuTR calculated from plasma [1-^{13}C]leucine enrichment than that of plasma α-[1-^{13}C]KIC enrichment was comparable with that of previous results of Sano et al. (2004) in sheep fed different levels of CP with an isoenergetic diet. Harris et al. (1992) used [^{3}H]- and [U-^{14}C]phenylalanine and [1-^{13}C]leucine isotopes to determine protein metabolism in growing sheep with different levels of dietary intakes, and stated that the PheTR and LeuTR increased significantly with increased dietary intake (300, 600 and 900 g grass pellets/day). Savary-Auzeloux et al. (2003) used a mixture of [U-^{13}C]amino acids in sheep at different levels of dietary intake and stated that plasma ILR increased with increased dietary intake and the absolute values of WBPF varied between amino acids. In another study, it has been reported that PheTR and LeuTR varied between fed and fasted sheep (Connell et al., 1997).

Protein synthesis and degradation

In the present experiment, plasma enrichment of α-[1-^{13}C]KIC was used to compare with the [$^2H_5$]phenylalanine model because the plasma α-[1-^{13}C]KIC to [1-^{13}C]leucine enrichment ratio was similar to data in a previous experiment in sheep (Sano et al., 2004), which explained that α-[1-^{13}C]KIC is the true precursor of intracellular leucine metabolism.

The numerical values of WBPS calculated from the plasma enrichment of the [$^2H_5$]phenylalanine in the present study were comparable with those of Fujita et al. (2006). Moreover, the values of WBPS and WBPD found by Harris et al. (1992) were also comparable with our results. Our findings are in agreement with their results. On the contrary, with a different isotope combination, Lobley et al. (2003) used [1-^{13}C]phenylalanine and [1-^{13}C]leucine to determine WBPS in sheep, fed 1.4 times of the maintenance energy intake at hourly intervals. They stated that the WBPS determined using the plasma enrichment of [1-^{13}C]leucine and [1-^{13}C]phenylalanine were similar within the study.

Although the WBPS and WBPD calculated from the [$^2H_5$]phenylalanine model were significantly lower than those of the [1-^{13}C]leucine method in the present study, the absolute values of WBPS and WBPD were comparable. In addition, the trend of WBPS and WBPD from M-diet to H-diet was apparently comparable between the [$^2H_5$]phenylalanine model and the [1-^{13}C]leucine method.

However, the values vary depending on the use of the respective amino acid contents in the carcass protein when calculating WBPS. In the present study the phenylalanine and leucine contents of body protein in sheep were assumed to be 0.035 and 0.066 g/g CP, respectively (Harris et al., 1992), during the calculation of WBPS. However, in another study, Connell et al. (1997) used 0.036 and 0.068 g/g CP for the phenylalanine and leucine concentrations of body protein in sheep, respectively. Harris et al. (1992) stated that the rate of protein synthesis based on leucine was higher than that based on phenylalanine from the same pool.

Whole body protein synthesis, calculated from plasma enrichment of the [$^2H_5$]phenylalanine tended to be higher, and that from α-[1-^{13}C]KIC was numerically higher for H-diet than for M-diet, which might be due to the higher intake of energy. Fujita et al. (2006) determined WBPS using the [$^2H_5$]phenylalanine model and found that WBPS increased with the increased dietary energy intake in adult goats. The WBPS was also enhanced with non-protein dietary intake in pigs (Reeds et al., 1981) and chicks (Kita et al., 1989). Moreover, in a previous study WBPS decreased numerically with increased CP intake in sheep fed isoenergetic diets (Sano et al., 2004).

Comparison of equations for calculating protein synthesis and degradation

In the [$^2H_5$]phenylalanine model the WBPS and WBPD were comparable between the equations described by Schroeder et al. (2006) and Thompson et al. (1989) which might be partially due to the proportion of carbon (C) oxidation contributing similarly to that of N excretion. However, for the [1-^{13}C]leucine method the higher values of WBPS and WBPD calculated using the equation described by Schroeder et al. (2006) than that of Krishnamurti and Janssens (1988) might be due to differences in determination of exhaled CO_2 and urinary N excretion.

CONCLUSION

Although WBPS and WBPD were lower for the [$^2H_5$]phenylalanine model, the absolute values and the dietary effects on WBPS and WBPD were comparable between the [$^2H_5$]phenylalanine model and the [1-^{13}C]leucine method in the present study. It is suggested that the [$^2H_5$]phenylalanine model could be an alternative to the [1-^{13}C]leucine method to determine WBPS and WBPD in sheep. Moreover, the equation described by Schroeder et al. (2006) could also be a better consideration for studying whole body protein metabolism in sheep because it does not need the measurement of oxidation even in the [1-^{13}C]leucine method and the results were also comparable within the study.

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