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Intermediary Metabolism of Plasma Acetic Acid, Glucose and Protein in Sheep Fed a Rice Straw-based Diet

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ABSTRACT : The present study was conducted to determine plasma acetate, glucose and protein metabolism using dilution of isotopes [[1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]leucine (Leu)] in sheep fed rice straw (*Oriza japonica* L.). Four sheep were assigned to either rice straw (RS-diet) or mixed hay (MH-diet) with a crossover design. Nitrogen (N) intake and N digestibility were lower ($p = 0.002$ and $p = 0.02$, respectively) for RS-diet than MH-diet, but N retention did not differ ($p>0.10$) between the diets. Concentrations of rumen acetate tended to be lower ($p = 0.07$), and propionate was higher ($p = 0.02$) for RS-diet than MH-diet. Concentrations of plasma lactate, non-esterified fatty acids, Leu and α-ketoisocaproic acid did not differ ($p>0.10$) between the diets, but plasma glucose and urea concentrations were lower ($p = 0.01$ and $p = 0.003$, respectively) for RS-diet than MH-diet. Turnover rate of plasma acetate did not differ ($p = 0.39$) between the diets, and plasma glucose and Leu turnover rates were numerically lower ($p = 0.15$ and $p = 0.14$, respectively) for RS-diet than MH-diet. Whole body protein synthesis and degradation did not differ ($p>0.10$) between the diets. Thus it can be concluded that the intermediary metabolism of acetate, glucose and protein on rice straw is comparable to mixed hay in sheep. (**Key Words :** Rice Straw, Intermediary Metabolism, Mixed Hay, Stable Isotope, Sheep)

INTRODUCTION

Agricultural by-product is one of the most important feed resources in sustainable animal production. Rice is the world's second largest cereal crop after wheat, and produces the largest amount of crop residue at about 330 million tons (Van Soest, 2006). Abundant availability of rice straw makes it important in animal production. Rice straw is used as animal feed in many countries of the world, but it is widely used in the dry summer of developing countries to raise livestock production. In south and south-east Asian countries, the potential use of rice straw as animal feed is particularly important as it constitutes the staple diet of ruminants. Despite the large quantities of rice straw available in rice-producing countries, it is not extensively used as animal feed due to lack of information of its effect on ruminant production. Since rice straw with low nutritive value and low digestibility is the basal feed for ruminants (Hossain et al., 2002), the nutritive value of rice straw and its effect on digestion attributes in small and large ruminants has been investigated (Acorda et al., 1992; Wu et al., 2005). Intermediary metabolism, particularly important for growth, lactation and production of ruminants, is influenced by dietary energy intake (Harris et al., 1992). However, information on intermediary metabolism in ruminants fed rice straw only is scanty. Therefore, it is necessary to know the effect of rice straw on intermediary metabolism of plasma nutrients in ruminants. The present study was designed to evaluate the effect of rice straw on the intermediary metabolism of plasma acetate, glucose and protein with simultaneous use of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]leucine (Leu) isotopes as well as on digestion attributes such as nitrogen (N) balance and rumen characteristics in sheep fed rice straw and mixed hay.

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MATERIALS AND METHODS

Animals, diets and management

Four crossbred (Corriedale×Suffolk) shorn sheep (*Ovis aries* L.), average age 3 yr and body weight (BW) 50±1 kg, were used in this experiment. The sheep were assigned to two dietary treatments; one rice straw (*Oriza japonica* L.) only diet (RS-diet) and the other a mixed hay diet (MH-diet) of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinaceae* L.) at a 60:40 ratio

Table 1. Chemical composition of feed

Item (%)	MH	RS
Dry matter	94.3	94.4
Crude protein	13.5	4.4
Crude ash	10.9	15.5
Neutral detergent fiber	69.7	73.8

MH = Mixed hay of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.).
RS = Rice straw (*Oriza japonica* L.).

(Table 1). The metabolizable energy (ME) was estimated at 1.30 kcal/g for rice straw (NARO, 2006) and 1.73 kcal/g for mixed hay (NRC, 1985). In the preliminary experiment 20% of the RS-diet remained as leftover and the MH-diet was completely consumed by the sheep when both diets were given at maintenance level. For this reason, feed allowance was 67.2 g/kg$^{0.75}$/d for RS-diet based on energy at maintenance level and 40.5 g/kg$^{0.75}$/d for MH-diet based on energy about 20% less than maintenance level to ensure similar energy intake for both diets. The experiment was performed using a crossover design with two 21-d periods. Two sheep were fed RS-diet during the first period and then fed MH-diet during the second period, and the other two sheep were fed these diets in the reverse order. The sheep were housed in individual pens in an animal barn during the first 14 d of each dietary period and were fed twice daily at 8:00 h and 20:00 h. Water was available *ad libitum*. On day 15, the sheep were moved to individual metabolism cages in a controlled environment chamber with an air temperature of 23±1°C and lighting from 7:00 h to 21:00 h. The sheep were weighed on days 1, 8, 15 and 21 of each dietary period. The handling of animals, including cannulation and blood sampling, was carried out according to the rules and regulations established by the Animal Care Committee of Iwate University.

Nitrogen balance test and collection of rumen fluid

A nitrogen balance trial was conducted for 5 days from d 16 to d 20 for each dietary treatment. Urine was collected from each sheep every 24 h in a bucket containing 50 ml of 6 N H_2SO_4 and the volume was recorded. A sub-sample of urine was stored at -30°C until analysis. Feces were also collected from each sheep every 24 h and dried at 60°C in a forced air oven for 48 h and then weighed after being held at room temperature for 5 days. The required amount of feces was ground to pass through a 1 mm screen and stored at room temperature for further analysis. On d 20, rumen fluid was collected before feeding (BF), 3 h (3F) and 6 h (6F) after feeding via a stomach tube for measuring pH, ammonia-N (NH_3-N) and volatile fatty acids (VFA). The pH of rumen fluid was measured immediately after collection with a pH meter (HM-10P, Toa Electronics Ltd., Japan). A sub-sample was centrifuged at 8,000×g for 10 min at 0°C (RS-18IV, Tomy, Japan), then 1 ml of supernatant was taken and mixed with 1 ml of 0.1 N HCl and stored at -30°C until measurement of rumen NH_3-N concentration. Residual rumen fluid samples were preserved at -30°C for further analysis.

Isotope dilution method

For determining the turnover rates (TR) of plasma acetate, glucose and Leu the isotope dilution methods using [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu were conducted simultaneously on d 21 of each dietary period. Two catheters, one for isotope infusion and another for blood sampling, were inserted into the left and right jugular veins on the morning of the study. The catheters were filled with sterile solution of tri-sodium citrate (0.13 mol/L) and at 12:00 h 87 μmol/kg$^{0.75}$ of [1-^{13}C]Na acetate (1-^{13}C, 99%, Cambridge Isotope Laboratories, Inc., USA), 3.1 μmol/kg$^{0.75}$ of [U-^{13}C]glucose (D-glucose -$^{13}C_6$, 99 atom % excess ^{13}C; Cambridge Isotope Laboratories, USA) and 7.2 μmol/kg$^{0.75}$ of [1-^{13}C]Leu (L-leucine-1-^{13}C, 99 atom % excess ^{13}C; Cambridge Isotope Laboratories, USA) dissolved in saline solution were injected as a priming dose through the jugular infusion catheter. Immediately after the injection of priming dose, the isotopes were continuously infused at rates of 87, 3.1 and 7.2 μmol/kg$^{0.75}$/h of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu, respectively, by a multichannel peristaltic pump (AC-2120, Atto Co. Ltd., Japan) for 4 h through the same catheter. Blood samples (5 ml) were collected through the sampling catheter just before the priming dose injection and at 30 min intervals during the last 2 h of the isotope infusion. The collected blood samples were immediately transferred to heparinized tubes, stored in crushed ice until centrifugation at 10,000×g for 10 min at 2°C, and the plasma samples then stored at -30°C for further analysis.

Chemical analysis

Analyses of proximate composition of the experimental diets were performed using the methods described in AOAC (1995). Nitrogen in diets, feces, urine and feed residues was analyzed by the Kjeldahl method with the Foss Kjeltec System (Tecator Digestor System and Kjeltec 2300, Foss Tecator, Sweden). Rumen VFA concentrations were determined by titrating the steam distillate of rumen fluid with 0.1 N NaOH. The titrated distillate was dried and then individual VFA were determined using gas chromatography (5890A, Hewlett Packard Co., USA). Ammonia-N content of rumen fluid was determined using a colorimetric method (Wetherburn, 1967).

Plasma [1-^{13}C]acetate enrichment and concentrations of plasma VFA and lactate were determined using selected ion monitoring with a gas chromatography-mass spectrometry system (GC/MS) (QP-2010, Shimadzu, Japan), according to

the procedure of Moreau et al. (2003) as previously described by Al-Mamun et al. (2009). Plasma [U-^{13}C]glucose enrichment was determined by the procedure of Tserng and Kalhan (1983) with slight modifications as described previously (Sano et al., 1996). The enrichment of [U-^{13}C]glucose was determined using selected ion monitoring with GC/MS. Concentrations of plasma glucose were determined using the method described by Huggett and Nixon (1957). Plasma amino acids and α-keto acids were separated and converted to N-methyl-N-t-butyl-dimethylsilyltrifluroacetamide (MTBSTFA) derivatives according to the procedures of Rocchiccioli et al. (1981) and Calder and Smith (1988), as described previously (Sano et al., 2004). Isotopic enrichments of plasma [1-^{13}C]Leu and α-[1-^{13}C]ketoisocaproic acid [α-[1-^{13}C]KIC] and concentrations of plasma Leu and α-KIC were measured by selected ion monitoring using the GC/MS. Concentrations of plasma non-esterified fatty acids (NEFA) and urea were determined using kits (NEFA C and Urea NB, Wako Pure Chemicals, Japan).

Calculation

For the isotope dilution method, the TR of plasma acetate, glucose and Leu were calculated using the equation given by Tserng and Kalhan (1983).

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

Where, TR is the turnover rate of plasma acetate, glucose and Leu, *I* is the infusion rate of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu and E is the plasma isotope enrichment of [1-^{13}C]acetate, [U-^{13}C]glucose and [1-^{13}C]Leu or α-[1-^{13}C]KIC at steady state.

Whole body protein synthesis (WBPS) and degradation (WBPD) were calculated from the relationships between whole body protein flux (WBPF), N absorption and urinary N excretion according to the equations described by Schroeder et al. (2006), as follows:

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

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

Leucine concentrations in carcass protein (66 g/kg) were used as described by Harris et al. (1992). Thus the WBPF was obtained by dividing the turnover rate of plasma Leu by 0.066.

Statistical analysis

All data were statistically analyzed with the MIXED procedure of SAS (1996). The least-squares means statement was used to test the effects of diet and period and the random effect was sheep. Results were considered significant at $p<0.05$ and a tendency was defined as $0.05 \leq p<0.10$. The repeated measures statement and the Tukey adjustment were used for the time course of changes and the significance level was $p<0.05$.

RESULTS

Body weight change did not differ ($p = 0.31$) between the diets. Dry matter intake (DMI) and estimated ME intake were higher ($p = 0.002$ and $p = 0.03$, respectively) for RS-diet than MH-diet. Nitrogen intake, N excretion through urine and feces and N digestibility were lower ($p<0.05$) for RS-diet than MH-diet, but N retention did not differ ($p = 0.39$) between the dietary treatments (Table 2).

Rumen pH was not affected ($p = 0.26$) by the diets, and decreased ($p<0.05$) after feeding (Table 3). Rumen NH_3-N concentration was lower ($p = 0.0002$) for RS-diet than MH-diet and decreased at 6F ($p<0.05$). The concentrations of rumen total VFA did not differ ($p = 0.17$) between the diets and also did not differ ($p = 0.28$) after feeding. The concentration of acetic acid tended to be lower ($p = 0.07$),

Table 2. Effect dietary intake on body weight change, dry matter intake, estimated metabolizable energy (ME) intake, nitrogen (N) balance and digestibility of N in sheep

Item	MH-diet	RS-diet	SEM	p-value
No. of sheep	4	4		
Body weight change (kg/d)	-0.11	-0.07	0.04	0.31
Dry matter intake (g/kg$^{0.75}$/d)	37	54	5	0.002
Estimated ME intake (kcal/kg$^{0.75}$/d)	63	70	3	0.03
N intake (g/kg$^{0.75}$/d)	0.85	0.47	0.12	0.002
N in faeces (g/kg$^{0.75}$/d)	0.28	0.21	0.02	0.01
N in urine (g/kg$^{0.75}$/d)	0.42	0.09	0.10	0.002
N absorption (g/kg$^{0.75}$/d)	0.57	0.26	0.10	0.004
N retention (g/kg$^{0.75}$/d)	0.15	0.17	0.02	0.39
N digestibility (%)	67	56	4	0.02

MH = Mixed hay of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.),
RS = Rice straw (*Oriza japonica* L.), SEM = Standard error of means.

Table 3. Dietary effects on rumen pH, concentrations of rumen ammonia-N and volatile fatty acids (VFA) before feeding (BF) and at 3h (3F) and 6h (6F) after feeding in sheep

Rumen parameters	Treatments						SEM	p-value		
	MH-diet			RS-diet				Diet	Time	Diet×Time
	BF	3F	6F	BF	3F	6F				
No. of sheep	4	4	4	4	4	4				
pH	6.9	6.8	6.7	7.0	6.8	6.9	0.10	0.26	0.04	0.04
NH_3-N (mg/dL)	8.4	9.6	7.4	2.3	1.5	1.0	2.2	0.0002	0.001	0.001
Total VFA (mmol/L)	91.8	92.5	98.3	88.0	89.9	89.2	2.1	0.17	0.28	0.56
Acetate (mmol/L)	69.7	69.8	73.8	62.9	59.5	62.2	3.2	0.07	0.40	0.84
Propionate (mmol/L)	14.9	14.6	17.1	18.1	23.2	19.7	1.9	0.02	0.26	0.10
iso-Butyrate (mmol/L)	0.9	0.9	0.7	0.5	0.4	0.5	0.1	0.04	0.18	0.44
Butyrate (mmol/L)	4.8	4.7	5.6	5.5	6.0	5.9	0.32	0.11	0.78	0.49
iso-Valerate (mmol/L)	1.2	1.6	0.7	0.6	0.4	0.5	0.3	0.08	0.29	0.22
Valerate (mmol/L)	0.4	1.0	0.4	0.4	0.4	0.4	0.1	0.20	0.13	0.25

MH = Mixed hay of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.),
RS = Rice straw (*Oriza japonica* L.), SEM = Standard error of means.

and the concentration of propionate was higher (p = 0.02) for RS-diet than MH-diet.

Concentrations of plasma glucose and urea were lower (p = 0.01 and p = 0.003, respectively) for RS-diet than MH-diet (Table 4). Concentrations of plasma acetate, Leu, NEFA and lactate did not differ (p>0.10) between the dietary treatments.

Plasma acetate concentration and enrichment of plasma [1-^{13}C]acetate remained constant during the last 2 h period of the [1-^{13}C]Na acetate infusion (data are not shown). Turnover rate of plasma acetate did not differ (p = 0.39) between the dietary treatments. Plasma glucose concentration and enrichment of plasma [U-^{13}C]glucose remained constant during the latter half of the

Table 4. Dietary effects on plasma acetate, glucose and protein metabolism and concentration of plasma non-esterified fatty acids (NEFA), urea and lactate in sheep

Item	MH-diet	RS-diet	SEM	p-value
No. of sheep	4	4		
Acetate				
Concentration (mmol/L)	0.363	0.410	0.047	0.48
TR (mmol/kg$^{0.75}$/h)	4.92	4.12	0.86	0.39
Glucose				
Concentration (mmol/L)	3.54	3.20	0.11	0.01
TR (mmol/kg$^{0.75}$/h)	1.58	1.37	0.15	0.15
Leu				
Concentration (μmol/L)	90.8	73.9	8.4	0.21
TR (mmol/kg$^{0.75}$/h)	0.259	0.205	0.027	0.14
WBPS (g/kg$^{0.75}$/h)	9.7	9.2	1.0	0.67
WBPD (g/kg$^{0.75}$/h)	8.8	8.1	1.0	0.58
α-KIC				
Concentration (μmol/L)	15.3	13.8	2.1	0.54
TR (mmol/kg$^{0.75}$/h)	0.338	0.250	0.039	0.16
WBPS (g/kg$^{0.75}$/h)	13.5	11.3	1.5	0.39
WBPD (g/kg$^{0.75}$/h)	12.5	10.3	1.5	0.37
NEFA concentration (mEq/L)	0.25	0.22	0.04	0.41
Urea concentration (mmol/L)	3.3	1.1	0.7	0.003
Lactate concentration (mmol/L)	0.352	0.404	0.066	0.16

MH = Mixed hay of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.), RS = Rice straw (*Oriza japonica* L.), SEM = Standard error of means, TR= Turnover rate, Leu = Leucine, WBPS = Whole body protein synthesis, WBPD = Whole body protein degradation, α-KIC = α-ketoisocaproic acid.

[U-^{13}C]glucose infusion (data are not shown). Turnover rate of plasma glucose was not affected (p = 0.15) by the dietary treatments. Concentrations of plasma Leu and α-KIC and enrichments of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC remained constant during the latter 2 h of the [1-^{13}C]Leu infusion (data are not shown). Plasma LeuTR calculated from [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichment was numerically lower (p = 0.14 and p = 0.16, respectively) for RS-diet than MH-diet, but WBPS and WBPD did not differ (p>0.10) between the diets.

DISCUSSION

The present experiment demonstrated that the effect of rice straw on intermediary metabolism of acetate, glucose and protein in sheep could be comparable to mixed hay. In the present study N digestibility and N excretion through urine was lower for RS-diet than MH-diet. This might be due to lower dietary CP intake for RS-diet, because similar results were previously found in lactating cows (Castillo et al., 2001) and sheep (Sano et al., 2004; Al-Mamun et al., 2008). The lower urinary N excretion for RS-diet, which suggested an increased urea recycling or a decreased protein oxidation due to lower CP intake than the MH-diet, is in agreement with Al-Mamun et al. (2008). Although N intake and urinary N excretion were lower for the RS-diet than MH-diet, N balance remained similar between the diets. This may suggests that the RS-diet contained more rumen undegradable protein than the MH-diet, and this result is in accordance with Al-Mamun et al. (2008).

In the present study, rumen pH was not affected by diets, but declined after feeding both diets. This drop in pH may be associated with the fermentation of carbohydrate and similar production of total VFA in the rumen. This is in agreement with the findings of Salman et al. (2008) who suggested that pH values were inversely related to total VFA concentration in the rumen of goats. The same trend was found in sheep by Santoso et al. (2006). The lower NH_3-N concentration in the rumen for RS-diet than MH-diet might be due to the lower dietary CP intake, because similar results were found in sheep (Al-Mamun et al., 2008). Although NH_3-N concentration was considerably lower for RS-diet than MH-diet, rumen pH did not differ between the diets in the present study.

Energy intake is inversely related to plasma NEFA concentrations (Sticker et al., 1995) and plasma NEFA is the best indicator of body lipid loss (Chillard et al., 2000). In the present study, unchanged plasma NEFA concentration for both diets might be due to use of roughage diets with restricted energy. Restricted energy intake for both diets caused similar mobilization of fatty acids from adipose tissue, which was responsible for similar BW loss of sheep on both diets. Lower plasma glucose concentration was found for RS-diet than MH-diet. Evans et al. (1974) also found a lower glucose concentration for a low quality than a high quality roughage diet.

The central role of rumen fermentation products in intermediary metabolism of ruminants is well recognized. In the present study, plasma acetate TR remained similar for both diets. This might be due to use of roughage diets which fermented equally in the rumen being responsible for similar plasma acetate concentration and TR. Prior (1978) determined plasma acetate TR in sheep fed restricted and *ad libitum* amounts of feed and stated that plasma acetate concentrations and the apparent turnover rate of acetate were not significantly influenced by level of feed intake. Plasma acetate TR in the present study was comparable with previous data as determined by [^{14}C]acetate dilution technique in sheep fed lucerne hay plus a concentrate mixture (Pethick and Lindsay, 1982; Sunagawa et al., 1986).

In adult sheep, plasma glucose TR was correlated with dietary energy intake level, suggesting that the nutritional status of the animal had at least as much influence as the supply of glucose precursors (Ortigues-Marty et al., 2003). The estimated energy intake level might also influence the dynamics of glucose metabolism as shown in lactating cows (Konig et al., 1984). In the present study, dietary treatments had no significant effect on plasma glucose TR, although propionate, a major glucose precursor in the ruminant, was higher for RS-diet than MH-diet. This might be due to roughage diets resulting in lower absorption of propionate, because Sano et al. (1999) suggested that, in sheep fed a roughage diet, propionate absorption is not strongly increased by feeding and gluconeogenesis is sustained by variable contributions of different precursors over the feeding cycle. Rodriguez et al. (1985) also reported that propionate infusion into the rumen failed to influence percentage of glucose derived from propionate, amount of propionate converted to glucose, and glucose TR in lactating goats fed a forage-based diet with concentrate mixture. The numerical value of plasma glucose TR in the present findings was comparable with data previously reported by Sano et al. (1999) in which plasma glucose TR was determined in sheep fed roughage diets at restricted and *ad libitum* amounts.

Plasma LeuTR in the present study was comparable to data reported previously in sheep (Sano et al., 2004). In the present study, numerically lower plasma LeuTR for RS-diet than MH-diet might be due to lower intake of CP, since dietary CP intake is positively correlated with LeuTR in sheep (Al-Mamun et al., 2008). However, Sano et al. (2004) reported that plasma LeuTR in sheep was influenced only marginally by dietary CP intake when ME intake was constant.

In the present study, even though plasma LeuTR differed slightly, WBPS and WBPD calculated from the enrichments of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC did not differ between the diets. In the equation used (Schroeder et al., 2006), lower urinary N excretion and lower N absorption result in higher protein synthesis (WBPS) and degradation (WBPD). In spite of different CP intake, WBPS and WBPD did not differ between the diets, which might be due to use of the above method of calculation. The values of WBPS and WBPD in this study were calculated from [1-^{13}C]Leu and α-[1-^{13}C]KIC, which were very similar to previous values calculated in sheep (Al-Mamun et al., 2007) using the same equation.

No significant differences were found in plasma acetate, glucose and protein metabolism in sheep between the dietary treatments. It can be suggested that the performance of RS-diet was comparable to MH-diet in relation to intermediary metabolism of plasma acetate, glucose and protein in sheep and hence rice straw could serve as an alternative feed for ruminants.

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