Effects of Rice Straw-based Diets on Kinetics of Plasma Nutrient Metabolism in Sheep

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Effects of Rice Straw-based Diets on Kinetics of Plasma Nutrient Metabolism in Sheep

A Thesis

Submitted in partial fulfilment of the requirements of the degree of

Doctor of Philosophy

by

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Under the supervision of

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Science of Bioproduction

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Dedication

I dedicate this thesis to my parents, brother and sisters, relatives, teachers, wife and daughter for their love, inspiration and continuous support.

Mohammad Khairul Alam

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Abbreviations

Abbreviated form		Elaborated form
AA	:	Amino acid
ADF	:	Acid detergent fiber
ADL	:	Acid detergent lignin
AOAC	:	Association of official analytical chemists
BW	:	Body weight
CF	:	Crude fiber
CP	:	Crude protein
d	:	Day
DM	:	Dry matter
FAO	:	Food and agriculture organization of the United Nations
FM	:	Fresh matter
GC	:	Gas chromatography
GC/MS	:	Gas chromatography/mass spectrometry
h	:	Hour
α-KIC	:	α-Ketoisocaproic acid
Leu	:	Leucine
ME	:	Metabolizable energy
MH	:	Mixed hay of orchardgrass and reed canarygrass
MTBSTFA	:	N-methyl-N-t-butyl-dimethylsilyltrifluoroacetamide
N	:	Nitrogen
Na	:	Sodium
NaOH	:	Sodium hydroxyde
NARO	:	National agriculture and food research organization
NDF	:	Neutral detergent fiber
NEFA	:	Non-esterified fatty acids
NH_3	:	Ammonia
NRC	:	National research council

RS	:	Rice straw
RSS	:	Rice straw supplemented with soybean meal
RSUM	:	Rice straw supplemented with urea and molasses
RSUMS	:	Rice straw supplemented with urea and molasses silage
SBM	:	Soybean meal
SEM	:	Standard error of the mean
TR	:	Turnover rates
VBN	:	Volatile basic nitrogen
VFA	:	Volatile fatty acids
WBPD	:	Whole body protein degradation
WBPF	:	Whole body protein flux
WBPS	:	Whole body protein synthesis

General Introduction

Background

Rice (*Oriza sativa*) is considered one of the most economically important cereal crops all over the world, serving as the daily basic source of nutrition for billions of people (Van Soest, 2006). Globally 155 million ha of rice is harvested annually and produces at about 596 million tons of straw (IRRI, 2001). Approximately 80% of the world's rice is grown by small-scale farmers in many developing countries and it is common to use rice straw as animal feed (Sarnklong et al., 2010).

In some parts of Asia rice straw is burned after harvest, incorporated into soil or used as a source of bio-energy (Binod et al., 2010; Li et al., 2010). However, in the mixed smallholder farming systems prevailing in large parts of South and South-East Asian countries, rice straw constitutes the most important feed resource for ruminants. Devendra and Thomas (2002) estimated that rice straw was the principal residue feed for over 90% of all livestock kept in this part of the world. The high utilization rates of rice straw as animal feed in Bangladesh and Thailand, where around 80% of the total available rice straw was used as livestock feed, making it the single most important feed resource for millions of farmers (Devendra and Sevilla, 2002). Moreover in China and Japan about 20-25% of total available rice straw is used as animal feed. In Japan utilization of whole crop rice silage is increasing day by day in dairy farming (Ogino et al., 2008), but in most tropical and sub-tropical countries, due to shortage of green roughage and alternative feed sources, the productivity of ruminants largely depends upon available crop residues, mainly rice straw (Baset et al., 2003). In dry summer

countries rice straw is especially important during the periods when other feeds are inadequate.

Limitations of rice straw as animal feed

Low crude protein content, low digestibility, high silica and lignin content are the major limitations of rice straw for use as animal feed. The high level of silica and lignin content of rice straw limits its voluntary intake by animals, impacting negatively on fiber digestibility and inhibiting degradability by rumen microbes (Mould, 2003; Van Soest, 2006; Frei et al., 2011). The combination of low voluntary intake, low digestibility and imbalanced mineral composition means that rice straw alone can not even meet the animal's maintenance needs. Therefore, presently many scientists are applying themselves to find out an effective way of utilizing rice straw for ruminant production.

Previous findings using rice straw

The supplementation of nitrogenous substrates has been reported to improve dry matter (DM) digestibility, feed intake and subsequently performance of animals (Han et al., 1993; Prasad et al., 1998; Vu et al., 1999). Warly et al. (1992) showed in a field trial that a ration of rice straw supplemented with soybean meal (SBM) increased both intake and degradability of dietary nitrogen (N). Some chemical substrates such as sodium hydroxide (NaOH), ammonia (NH_3) and urea are used to improve the utilization of rice straw, because these substances can be absorbed into the cell wall and chemically break down the ester bonds between hemicellulose and cellulose, and physically makes the structural fibers swell (Lam et al., 2001). This process enables the rumen

microorganisms to attack the structural carbohydrates, enhancing degradability and palatability of the rice straw (Shen et al., 1999; Selim et al., 2004). Currently, urea is widely used rather than NaOH or NH_3, because it is safe to use and can be obtained easily in many developing countries. Supplementation of urea in combination with molasses to rice straw improved the daily gain, DM intake, digestibility, ruminal characteristics compared to only rice straw (Acorda et al., 1992; Barnah et al., 1992; Baset et al., 2003).

Research on rice straw silage

At present in many countries it is normal practice to use rice straw-based silage, because ensilation is the method of improving the feed quality and conserving rice straw for animal feed. In recent years, Japanese and Korean studies showed an improvement in feeding value of rice straw by ensiling (Cai, 2006). However, successful ensiling of rice straw is difficult due to its hollow stem, low water soluble carbohydrates and less epiphytic bacteria (Li et al., 2010). In order to improve the quality of rice straw silage, some commercial lactic acid bacteria, sources of N and soluble carbohydrate are added as additives for ensiling. Many scientists showed that the ensiling of rice straw supplemented with urea and molasses improved the digestibility, palatability and nutritional values of straw due to fermentation during the ensiling period (Yulistiani et al., 2003; Fadel Elseed, 2004; Hue et al., 2008).

Hypothesis

It can be expected that rice straw supplemented or treated with protein and energy sources will be improved from rice straw alone, and intermediary metabolism of plasma

nutrients will be influenced through providing the easily fermentable nitrogenous substrates and energy substrates for increasing the microbial activities.

Objectives

However, to date, the animal nutritionists have focused their research on the effects of rice straw-based diets on voluntary intake, digestive function and ruminal characteristics in ruminants, and no research has been conducted to investigate the feeding effects of rice straw-based diets on intermediary metabolism of plasma nutrient kinetics in ruminants. From this point of view the present research was designed to evaluate the feeding effects of rice straw-based diets on digestive function and intermediary metabolism of plasma nutrients in sheep using stable isotope dilution methods.

Experimental layout

To know the performance of rice straw-based diets on digestive function and intermediary metabolism of plasma nutrients, four several experiments were conducted during my research tenure. First of all, experiment-1 (**Chapter1**) was conducted to evaluate the feeding effects of only rice straw, experiment-2 (**Chapter2**) was conducted to determine the performance of rice straw supplemented with SBM, experiment-3 (**Chapter3**) was conducted to investigate the effects of rice straw supplemented with urea and molasses, and finally experiment-4 (**Chapter4**) was conducted to know the effects of ensiling rice straw supplemented with urea and molasses. It is hoped that the present findings will provide information about means of improving rice straw use for raising ruminant production.

Chapter1

Intermediary Metabolism of Plasma Acetate, Glucose and Leucine Kinetics in Sheep Fed Rice Straw

Introduction

Agricultural by-products are one of the most important feed resources in sustainable animal production. Abundant availability of rice straw makes it important in animal production (FAO, 1998, Van Soest, 2006). Due to shortage of alternative feed sources, rice straw is widely used in the dry summer and 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. The nutritive value of rice straw and its effect on digestion attributes in small and large ruminants has been investigated (Acorda et al., 1992; Hossain et al., 2002; Wu et al., 2005). However, no research has been conducted on intermediary metabolism in ruminants consuming rice straw. Thus the present study was designed to evaluate the effect of rice straw on the intermediary metabolism of plasma acetate, glucose and leucine (Leu) in sheep using isotope dilution methods of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu, as well as digestion attributes such as N balance and rumen characteristics in sheep compared with mixed hay.

Materials and Methods

Animals, diets and management

Four crossbred (Corriedale x Suffolk) shorn sheep (*Ovis aries*) average age three years and having body weight (BW) of 50.1±1.1 kg were used in this experiment. The

sheep were assigned to two dietary treatments; one is rice straw (*Oriza japonica*) only (RS-diet) and the other is mixed hay (MH-diet) of orchardgrass (*Dactylis glomerata*) and reed canarygrass (*Phalaris arundinaceae*) at a 60:40 ratio (**Table 1.1**). 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% RS-diet remained as leftover and 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 the RS-diet based on energy at maintenance level and 40.5 $g/kg^{0.75}/d$ for the MH-diet based on energy about 20% less from maintenance level to ensure the almost same energy intake for both diets. The experiment was performed using a crossover design with two 21 d periods. Two sheep were fed the RS-diet during the first period and then fed the MH-diet during the second period, and the other two sheep were fed in the reverse order. The sheep were housed in individual pens in an animal barn during the first 14 d of each dietary period. The sheep were fed at 8:00 h and 20:00 h and fresh tap water was available *ad libitum*. On day 15, the sheep were moved to individual metabolic cages in a controlled environment chamber at an air temperature of 23±1 °C with lighting from 7:00 h to 21:00 h. The sheep were weighed on d 1, d 8, d 15 and d 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. The experimental layout is shown in **Figure 1.1**.

Table 1.1. Chemical composition of experimental feed on air dry matter basis

Items (%)	Mixed hay	Rice straw
Dry matter	94.3	94.4
Crude protein	13.5	4.4
Crude Ash	10.9	15.5
Crude fiber	29.6	32.0
NDF	70.0	74.0
ADF	33.0	42.0
ADL	1.9	2.4

NDF = Neutral detergent fiber, ADF = Acid detergent fiber,
ADL = Acid detergent lignin

Nitrogen balance

A nitrogen balance trial was conducted for 5 d (from d 16 to d 20) of each dietary treatment. Urine was collected from each sheep every 24 h in a plastic bucket containing 50 ml of 6 N H_2SO_4 solution to prevent N loss. The urine was shaken properly after keeping record of total volume, and then sub-samples (50 mL) were 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 placed at room temperature for 5 d. Then the air dried samples were weighed and sub-samples were ground to pass through a 1 mm screen and stored at room temperature for further analysis.

Collection of rumen fluid

On d 20, rumen fluid was collected at 0, 3 and 6 h after feeding via a stomach tube for measuring the pH, ammonia (NH_3) and volatile fatty acids (VFA). The pH of the 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 an aliquot (1 mL) of supernatant was acidified with 1 mL of 0.1 N HCl and stored at -30 °C for measuring the rumen NH_3 concentration. The residuals of rumen fluid were also preserved at -30 °C for further analysis.

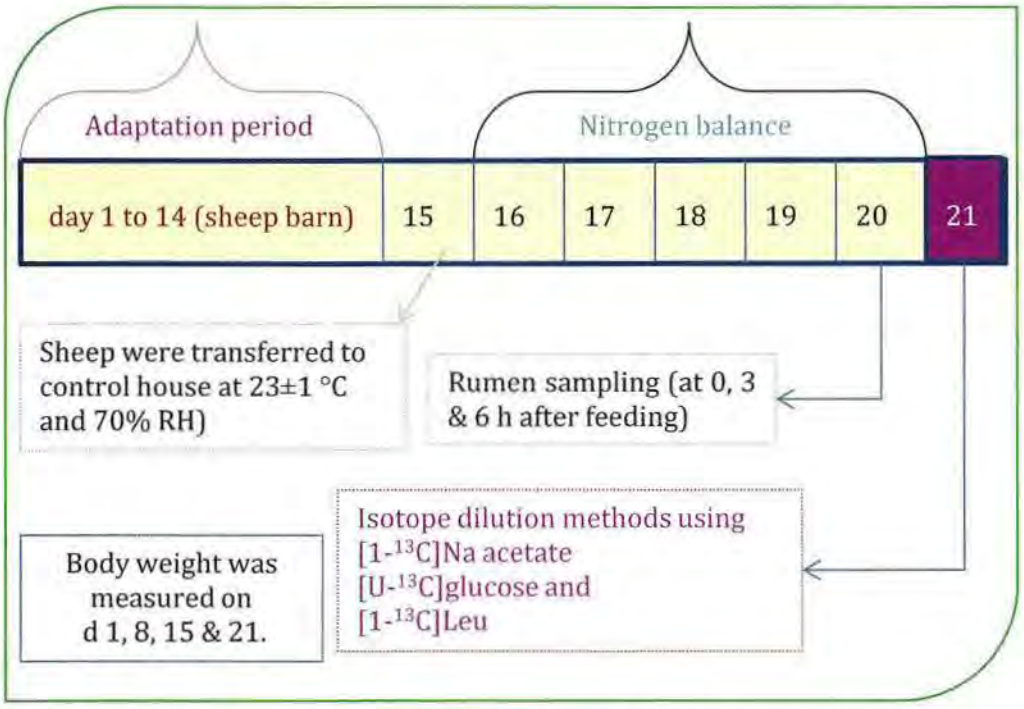

Figure 1.1. Schematic diagram of experimental layout showing the sampling protocols

Isotope dilution method

For determining the turnover rates (TR) of plasma acetate, glucose and Leu an isotope dilution method using [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu was 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 isotope dilution study. The catheters were filled with sterile solution of tri-sodium citrate (0.13 mol/L). At 12:00 h on the day of the isotope dilution method, 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 priming dose through the jugular infusion catheter. Immediately after the priming dose injection the isotopes were continuously infused at rates of 87, 3.1 and 7.2 μmol/kg$^{0.75}$/h for [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 (**Figure 1.2**). Blood samples (10 mL) were collected through the sampling catheter just before of the priming dose injection and (5 mL) every 30 min intervals during the last 2 h of the isotope dilution study. The collected blood samples were immediately transferred to heparinized tubes and stored in crushed ice until centrifugation. Blood samples were centrifuged at 10,000 × *g* for 10 min at 2 °C and the plasma samples were then stored at -30 °C for further analysis.

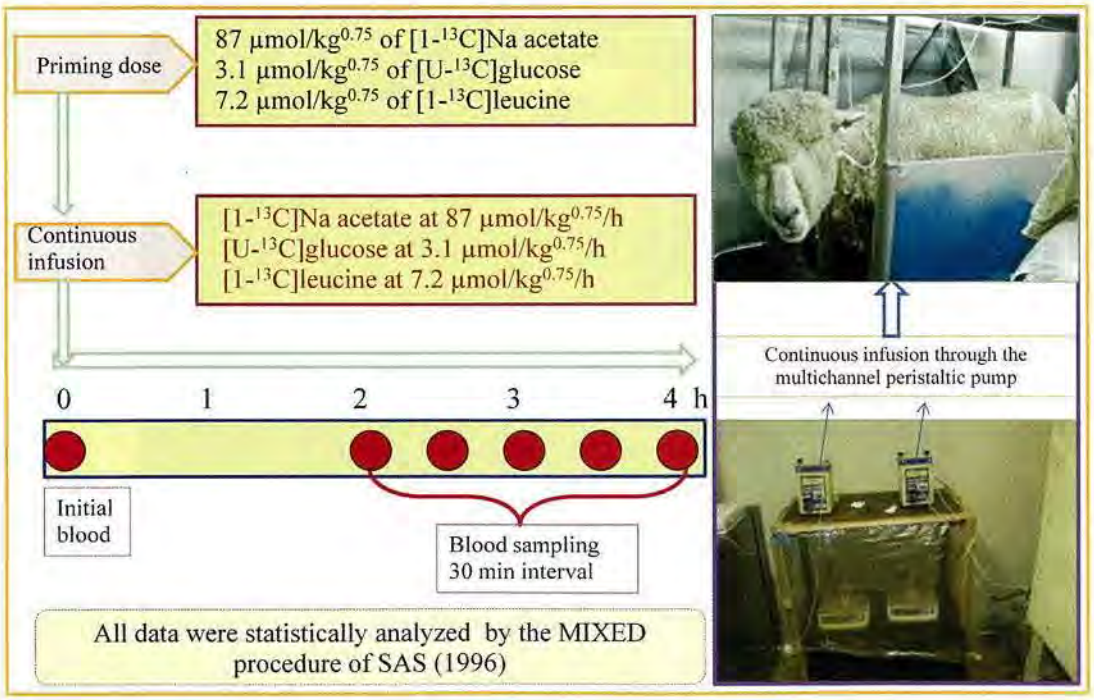

Figure 1.2. Layout of isotope infusion and blood sample collection method

Chemical analysis

Analyses of proximate components of the experimental diets were performed using the methods described in AOAC (1995). Nitrogen in diets, feces, urine and also the leftover of the given diets was analyzed by the Kjeldahl method with the Foss Kjeltec System (Tecator Digestor System and Kjeltec 2300, Foss Tecator, Sweden) shown in **Figure 1.3.** 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 the individual VFA were determined using the gas chromatography (GC) (5890A, Hewlett Packard Co., USA). Ammonia-N content of rumen fluid was determined using the colorimetric method (Weatherburn, 1967).

Figure 1.3. Schematic diagram of Foss Kjeltec System (Tecator Digestor System and Kjeltec 2300, Foss Tecator, Sweden) used for determinig the N balance

Concentrations of plasma free amino acid (AA), NH_3 and urea were determined using an automated AA analyzer (JLC-500/V, JEOL, Japan). Concentration of plasma non-esterified fatty acid (NEFA) was determined enzymatically using a diagnostic kit (NEFA C, Wako Pure Chemicals, Japan).

Plasma [1-^{13}C]acetate enrichment and concentrations of plasma VFA and lactate were determined using the selected ion monitoring system with the gas chromatography mass spectrometry system (GC/MS) (QP-2010, Shimadzu, Japan) after converting to N-methyl-N-t-butyl-dimethylsilyltrifluroacetamide (MTBSTFA) derivatives 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 modification as described previously (Sano et al., 1996). The enrichment of [U-^{13}C]glucose was determined using the selected ion monitoring system with the GC/MS. Concentrations of plasma glucose determined using the method described by Huggett and Nixon (1957).

Plasma AA and α-keto acids were separated and converted to 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 the selected ion monitoring method using the GC/MS.

Calculation

Mean values with standard error of the mean (SEM) are given. For the isotope dilution method, the TR of plasma acetate, glucose and Leu were calculated using the equation given by Tserng and Kalhan (1983) as follows:

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

Where, 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. Plasma Leu TR model is shown in **Figure 1.4.**

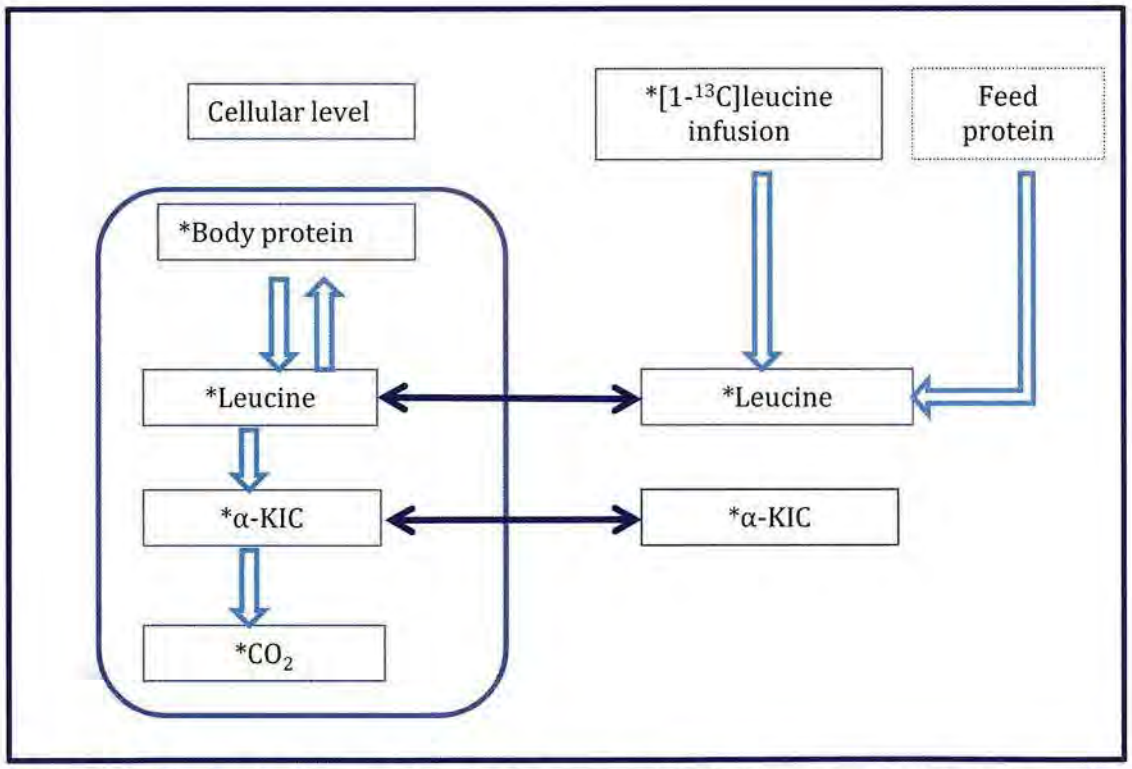

Figure 1.4. Schematic diagram of turnover rate model of plasma Leu showing plasma Leu and its derivative of α-KIC enrichment

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

WBPF = LeuTR/0.066

WBPS = WBPF - (urinary N × 6.25)

WBPD = WBPF - (absorbed N × 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 square means statement was used to test the effects of diet and period. The random effect was sheep. Results were considered significant at the $P < 0.05$ level and a tendency was defined as $0.05 \leq P < 0.10$. The repeated statement and the Tukey adjustment were used for the time course of changes and the significance was $P < 0.05$.

Results

Daily profile and N balance

Body weight change did not differ between diets (**Table 1.2**). Dry matter intake and estimated ME intake were higher ($P = 0.002$ and $P = 0.03$, respectively) for the RS-diet than the MH-diet. Nitrogen intake, N excretion through feces, N excretion through urine

and N digestibility were lower ($P < 0.05$) for the RS-diet than the MH-diet, and N retention did not differ between dietary treatments.

Table 1.2. Dietary effects on body weight (BW) change, dry matter (DM) intake, estimated metabolizable energy (ME) intake, nitrogen (N) balance and N digestibility in sheep

Items	MH-diet	RS-diet	SEM	*P*-value
No. of sheep	4	4		
BW change (kg/d)	-0.11	-0.07	0.04	0.31
DM intake (g/kg$^{0.75}$/d)	37	54	5	0.002
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 feces (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 and reed canarygrass, RS = Rice straw,
SEM = Standard error of the mean.

Rumen fermentation characteristics

Rumen pH did not differ between diets and decreased ($P < 0.05$) after feeding (**Table 1.3**). Rumen NH_3 concentration was lower ($P = 0.0002$) for the RS-diet and decreased ($P < 0.05$) at 6 h after feeding. The concentrations of rumen total VFA did not differ between diets, and also did not change after feeding. Acetate concentration in the rumen tended to be lower ($P = 0.07$) for the RS-diet than the MH-diet. Concentration of rumen propionate was higher ($P = 0.02$), iso-butyrate was lower ($P = 0.04$) and iso-valerate tended to be lower ($P = 0.08$) for the RS-diet than the MH-diet, but valerate did not differ between diets.

Table 1.3. Dietary effects on rumen pH, concentrations of rumen ammonia (NH_3) and volatile fatty acids (VFA) at 0, 3 and 6 h after feeding

Items	Treatments						SEM	*P*-value		
	MH-diet			RS-diet						Diet
	0	3	6	0	3	6		Diet	Time	×
No. of sheep	4	4	4	4	4	4				Time
pH	6.88[a]	6.83[a]	6.69[b]	6.99	6.76	6.90	0.10	0.26	0.04	0.04
(mmol/L)										
NH_3	4.94[b]	5.64[a]	4.33[c]	1.33[d]	0.89[e]	0.60[f]	1.2	0.0002	0.001	0.001
Total VFA	91.8	92.5	98.3	88.0	89.9	89.2	2.1	0.17	0.28	0.56
Acetate	69.7	69.8	73.8	62.9	59.5	62.2	4.7	0.07	0.40	0.84
Propionate	14.9	14.6	17.1	18.1	23.2	19.7	1.4	0.02	0.26	0.10
Iso-Butyrate	0.91	0.86	0.69	0.49	0.38	0.49	0.09	0.04	0.18	0.44
Butyrate	4.8	4.7	5.6	5.5	6.0	5.9	0.66	0.11	0.78	0.49
Iso-Valerate	1.2	1.6	0.72	0.59	0.39	0.50	0.22	0.08	0.29	0.22
Valerate	0.38	0.96	0.36	0.36	0.40	0.38	0.13	0.24	0.13	0.25

MH = Mixed hay of orchardgrass and reed canarygrass,
RS = Rice straw, SEM = Standard error of the mean,
[a,b,c] Values on the MH-diet with different superscripts differ ($P < 0.05$),
[d,e,f] Values on the RS-diet with different superscripts differ ($P < 0.05$).

Plasma metabolites

Plasma total and almost all free AA concentrations determined with the pre-infusion of isotope dilution method were significantly lower ($P < 0.05$) for the RS-diet compared to the MH-diet (**Table 1.4**). Concentrations of plasma NH_3 and urea were lower ($P < 0.05$) for the RS-diet than the MH-diet. Concentrations of plasma NEFA and lactate did not differ between diets.

Table 1.4. Dietary effects on plasma metabolite concentrations in sheep

Items	MH-diet	RS-diet	SEM	*P*-value
No. of sheep	4	4		
Essential AA (μmol/L)				
Threonine	259	130	35	0.0001
Valine	218	175	18	0.03
Methionine	37	15	6	0.02
Iso-leucine	104	67	10	0.04
Leucine	126	76	14	0.07
Phenylalanine	52	36	5	0.08
Histidine	37	22	4	0.01
Lysine	34	25	4	0.02
Nonessential AA (μmol/L)				
Aspartic acid	14	13	2	0.55
Serine	234	167	21	0.06
Asparagine	105	64	13	0.004
Glutamic acid	253	142	25	0.05
Glutamine	120	82	13	0.14
Glycine	690	463	68	0.04
Alanine	198	178	9	0.06
Tyrosine	79	59	9	0.09
Tryptophan	177	107	26	0.01
Arginine	126	86	16	0.07
Proline	76	56	10	0.001
Total AA (μmol/L)	2843	2160	239	0.01
NH_3 (μmol/L)	389	182	72	0.03
Urea (mmol/L)	6.86	2.42	1.60	0.01
NEFA (μEq/L)	254	222	36	0.41
Lactate (μmol/L)	352	404	66	0.16

MH = Mixed hay of orchardgrass and reed canarygrass, RS = Rice straw, SEM = Standard error of the mean, AA = Amino acid, NEFA = Non-esterified fatty acid.

Plasma acetate, glucose and Leu kinetics

Plasma acetate concentration and enrichment of plasma [1-^{13}C]acetate remained constant during the last 2 h of the [1-^{13}C]Na acetate infusion (**Figure 1.5**). Concentration of plasma acetate determined in the latter half of the primed-continuous infusion of isotope dilution method did not differ between diets. (**Table 1.5**). Turnover rate of plasma acetate calculated from the enrichment of [1-^{13}C]acetate did not differ between the RS-diet and the MH-diet.

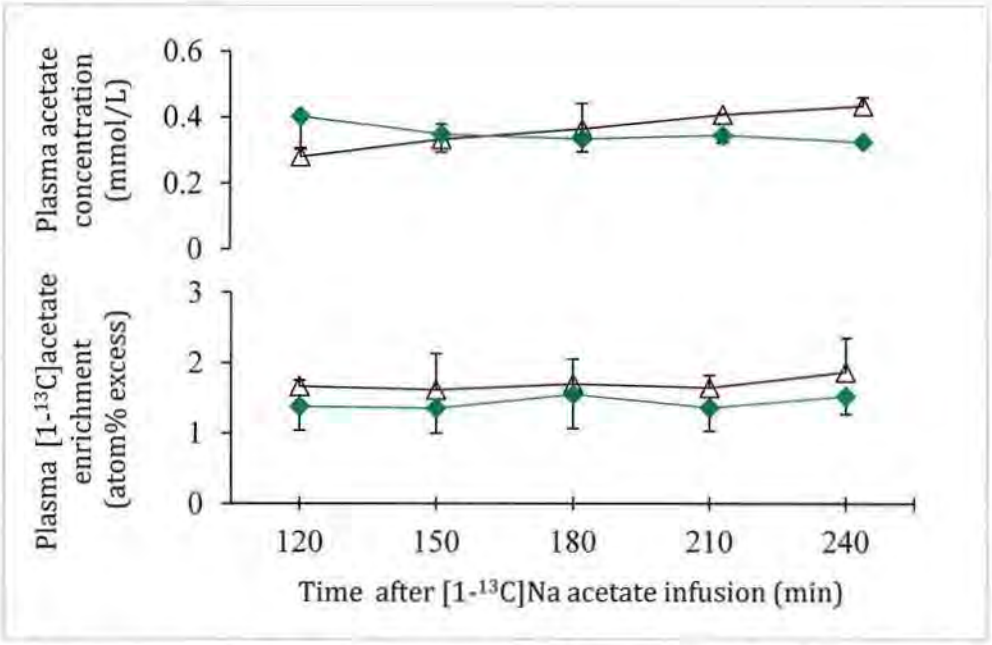

Figure 1.5. Time course changes in plasma acetate concentration and plasma enrichment of [1-^{13}C]acetate during the last 2 h continuous infusion of [1-^{13}C]Na acetate in sheep fed the RS-diet (Δ) and the MH-diet (♦). (Means ± SEM).

Plasma glucose concentration and enrichment of plasma [U-^{13}C]glucose were stable during the latter half of the [U-^{13}C]glucose infusion (**Figure 1.6**). Concentration of plasma glucose calculated from the latter half of the primed-continuous infusion of

isotope dilution method was lower ($P = 0.01$) for the RS-diet compared to the MH-diet. Plasma glucose TR calculated from plasma [U-^{13}C]glucose enrichment did not differ between diets (**Table 1.5**).

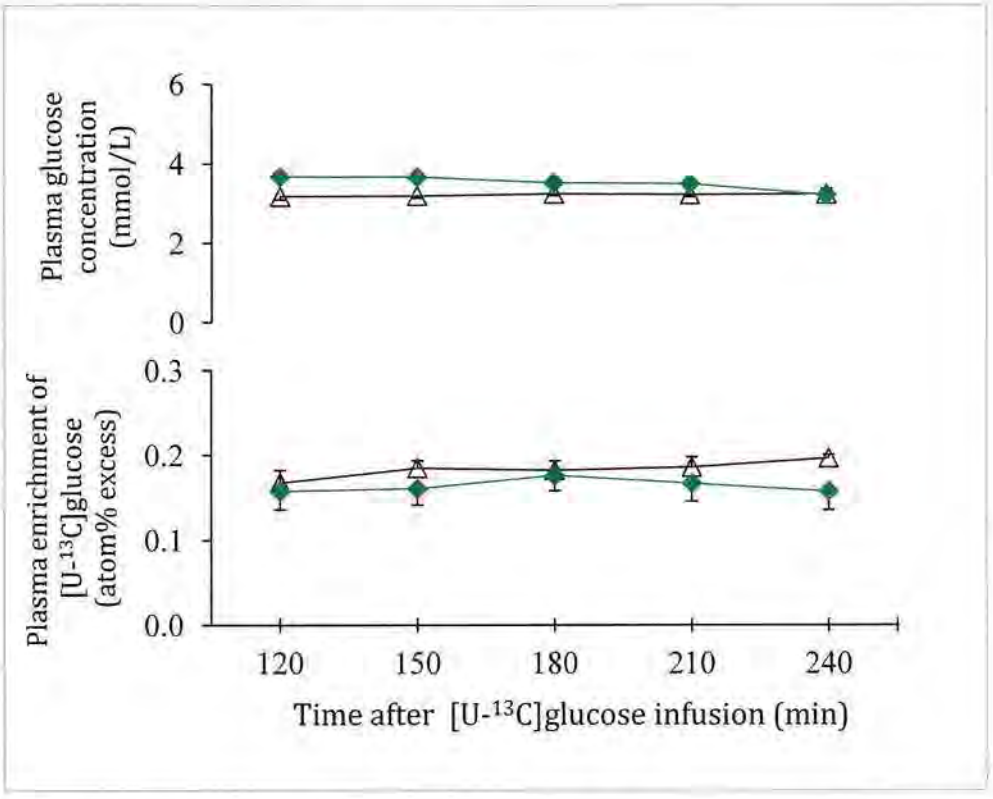

Figure 1.6. Time course changes in plasma glucose concentration and plasma enrichment of [U-^{13}C]glucose during the last 2 h continuous infusion of [U-^{13}C]glucose in sheep fed the RS-diet (Δ) and the MH-diet (♦). (Means ± SEM).

Concentration of plasma Leu and enrichment of plasma [1-^{13}C]Leu remained constant during the last 2 h of [1-^{13}C]Leu infusion (**Figure 1.7**). Plasma α-KIC concentration and enrichment of α-[1-^{13}C]KIC were also stable during the latter half of [1-^{13}C]Leu infusion (**Figure 1.8**). Plasma LeuTR as well as WBPS and WBPD determined from the enrichments of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC did not differ between dietary treatments (**Table 1.5**).

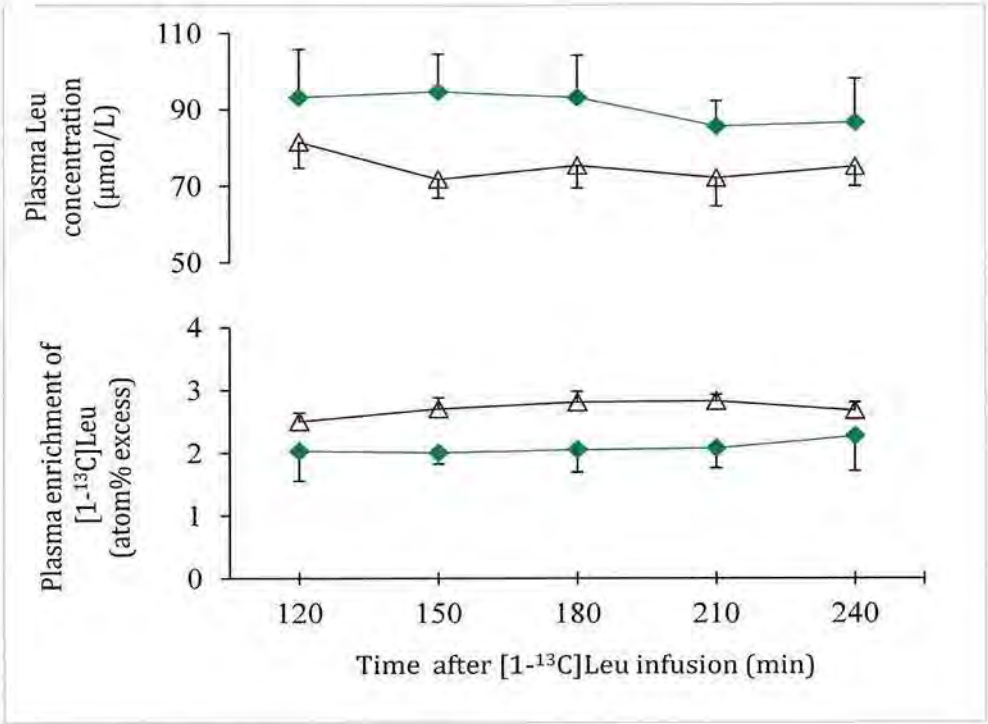

Figure 1.7. Time course changes in plasma Leu concentration and plasma enrichment of [1-^{13}C]Leu during the last 2 h continuous infusion of [1-^{13}C]Leu in sheep fed the RS-diet (Δ) and the MH-diet (♦). (Means ± SEM).

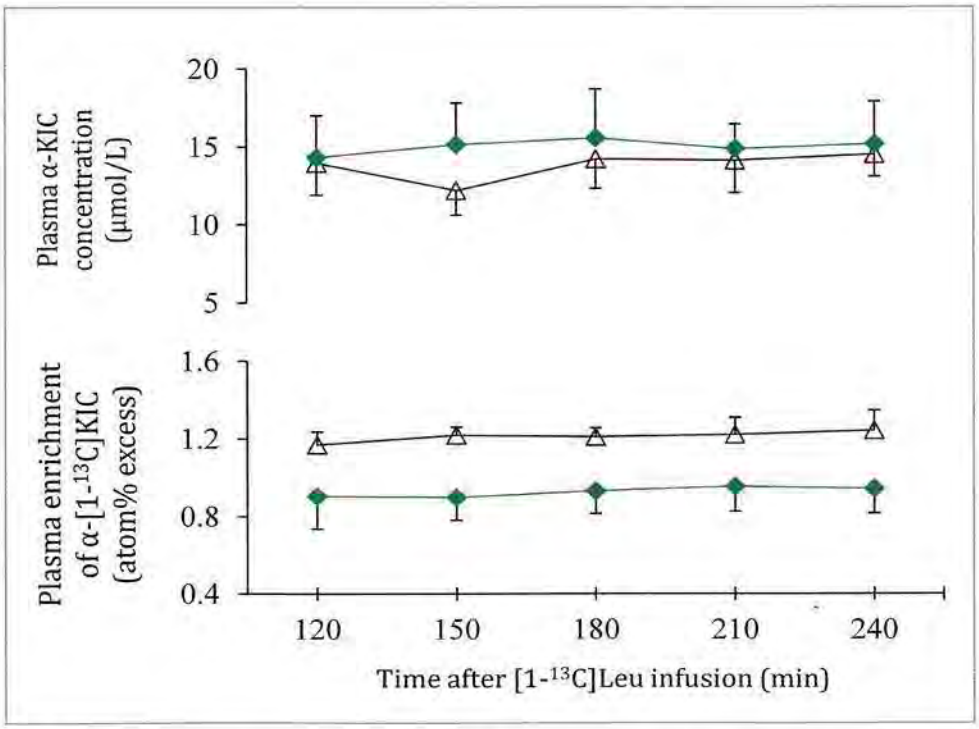

Figure 1.8. Time course changes in plasma α-KIC concentration and plasma enrichment of α-[1-^{13}C]KIC during the last 2 h continuous infusion of [1-^{13}C]Leu in sheep fed the RS-diet (Δ) and the MH-diet (♦). (Means ± SEM).

Table 1.5. Dietary effects on plasma acetate, glucose and leucine (Leu) metabolism in sheep

Items	MH-diet	RS-diet	SEM	*P*-value
No. of sheep	4	4		
Acetate				
Concentration (μmol/L)	363	410	47	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	0.20	0.15
Leu				
Concentration (μmol/L)	90.8	73.9	8.4	0.21
TR (μmol/kg$^{0.75}$/h)	259	205	27	0.14
WBPS (g/kg$^{0.75}$/d)	9.7	9.2	1.0	0.67
WBPD (g/kg$^{0.75}$/d)	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)	338	251	39	0.16
WBPS (g/kg$^{0.75}$/d)	13.5	11.3	1.5	0.39
WBPD (g/kg$^{0.75}$/d)	12.5	10.3	1.5	0.37

MH = Mixed hay of orchardgrass and reed canarygrass, RS = Rice straw, SEM = Standard error of the mean, TR = Turnover rate, WBPS = Whole body protein synthesis, WBPD = Whole body protein degradation, α-KIC = α-ketoisocaproic acid.

Discussion

The present study demonstrates that rice straw alone is not sufficient for raising ruminant production, because, even though estimated ME intake was greater for the RS-diet, turnover rates of plasma acetate, glucose and Leu as well as WBPS and WBPD were numerically lower for the RS-diet compared to the MH-diet.

Nitrogen balance

The considerably lower urinary N excretion for the RS-diet suggested a decreased protein oxidation due to lower CP intake than the MH-diet. This is in agreement with

the results found in sheep by Al-Mamun et al. (2008), and in lactating cows by Castillo et al. (2001). Although N intake was lower for the RS-diet than the MH-diet, N retention did not differ between diets. This may be due to recycle of N as urea from liver to rumen via saliva. Because, recycling of blood urea to the rumen allows ruminant to survive on diets very low in N (Kohn et al., 2005). Considerably lower N digestibility for the RS-diet than the MH-diet might be due to lower dietary N intake for the RS-diet. The present result was supported by Sano et al. (2004), who showed the lower N digestibilty in sheep for low CP intake diet than medium and high CP intake diet.

Rumen fermentation characteristics

In the present study rumen pH was not affected by the diets, but declined after feeding for both diets. This drop in pH may be associated to 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 concentration in the rumen for the RS-diet might be due to lower dietary CP intake. A similar response was observed in sheep in the previous findings (Al-Mamun et al., 2008).

Plasma metabolites

Several factors are responsible to influence the level of AA in blood such as absorption, tissue utilization and liver catabolism, dietary types and frequency of feeding (Young et al., 1973, Cecava et al., 1990, Lapierre and Lobley, 2001). In the present study considerably lower N intake for the RS-diet resulted in lower plasma free

AA concentrations in sheep. Lower plasma free AA concentrations in the present study might be due to inadequate supply of dietary protein in accordance with Pendlum et al. (1980) and Lobley et al. (1987). Plasma NH_3 concentration is positively correlated to production of NH_3 in the rumen and absorption into portal blood (Lobley et al., 1995). In the current study lower plasma NH_3 concentration for the RS-diet indicated the lower absorption of NH_3 from the rumen. Plasma NEFA concentration is the best indicator of body lipid loss (Chilliard et al., 2000). Energy intake is inversely related to plasma NEFA concentration (Sticker et al., 1995). The similar NEFA concentration for both diets in this study might be due to use of roughage diets with restricted energy. Restricted energy intake for both diets caused similar fatty acid mobilization from adipose tissue which was responsible for similar BW loss of sheep for both diets.

Plasma acetate, glucose and Leu kinetics

The central role of rumen fermentation products in intermediary metabolism of ruminants is well recognized. Acetate is the primary fatty acid produced in the rumen, absorbed through rumen wall and then to the portal vein (Bergman, 1990; Quigley et al., 1991; Sutton et al., 2003). That means plasma acetate turnover rate depends largely on dietary type, intake, microbial activities and dietary carbohydrate fermentation for acetate production in the rumen and absorption into the portal vein. In the present study plasma acetate TR did not differ between diets, but numerical values were slightly lower for the RS-diet than the MH-diet. This might be due to poor fermentability of rice straw in the rumen, resulting in a tendency of lower acetate concentration for the RS-diet. Similarly, Prior (1978) determined plasma acetate TR in sheep fed restricted and *ad*

libitum amounts of feed and did not find any significant difference between diets. Numerical values of plasma acetate TR in the present study were comparable with the data previously determined by using [^{14}C]acetate dilution technique in sheep fed lucerne hay plus concentrate mixture (Sunagawa et al., 1986).

Lower plasma glucose concentration was found for the RS-diet than the MH-diet. Evans et al. (1974) also found lower glucose concentration for a low quality roughage diet than a high quality roughage diet. 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 (Konig et al., 1984; Ortigues-Marty et al., 2003). In the present study plasma glucose TR basically did not differ between diets, but numerical values were slightly lower for the RS-diet than the MH-diet, although propionate, a major glucose precursor in the rumen, was higher for the RS-diet than the MH-diet. This might be due to roughage diets resulting 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 the percentage of glucose derived from propionate, the amount of propionate converted to glucose and glucose TR in lactating goats fed a forage based diet with concentrate mixture. The numerical values of plasma glucose TR in the present findings were comparable with the data previously reported in sheep fed roughage diets at restricted and *ad labitum* amounts (Sano et al., 1999).

Plasma LeuTR in the present study was comparable to data reported previously in sheep (Sano et al., 2004). In the present study plasma LeuTR calculated from [1-^{13}C]Leu infusion rate and the enrichments of both plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC did not differ between diets, but numerical values were slightly lower for the RS-diet than the MH-diet. This might be due to lower intake of CP for the RS-diet, because 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 slightly by dietary CP intake when ME intake was constant. Although no significant difference was found, the numerical values of WBPS and WBPD calculated from plasma LeuTR using N balance were slightly lower for the RS-diet compared to the MH-diet. In the equation used (Schroeder et al., 2006), lower urinary N excretion and lower N absorption results in higher protein synthesis and degradation as described previously (Sano et al., 2004). In spite of different CP intake the WBPS and WBPD did not differ between diets. This variation might be due to greater estimated ME intake for the RS-diet than the MH-diet. This is in agreement with the obsevation of Lapierre et al. (2002). Moreover, the values of WBPS and WBPD in this study calculated from [1-^{13}C]Leu and α-[1-^{13}C]KIC, were very similar with the values reported in sheep (Al-Mamun et al., 2007) calculated using the same equation. From the present results it was found that the turnover rates of plasma acetate, glucose and Leu as well as WBPS and WBPD were numerically lower for the RS-diet than the MH-diet, even though estimated ME intake was higher for the RS-diet than the MH-diet. Considering the whole results of the current study it could be proven that by using only rice straw for

ruminants, satisfactory output is not possible because of its low voluntary intake and low digestibility. Further research should be done to find out the effect of rice straw supplemented with protein or energy source on intermediary metabolism of plasma nutrients in sheep.

Chapter2

Intermediary Metabolism of Plasma Acetate and Rumen Fermentation Characteristics in Sheep Fed Rice Straw Supplemented with Soybean Meal

Introduction

From the previous study (**Chapter1**) it was understood that low CP content, low digestibility and low voluntary intake were the main limiting factors in utilization of rice straw only as feed to support animal production. It is necessary to recover the CP deficiency of rice straw to get better performance in ruminant production. Soybean meal is highly degradable CP source with high energy content, and supplementation of SBM to rice straw improved the efficiency of gain, digestive function and consumption of straw by heifers and sheep (Church and Santos, 1981; Wu et al., 2005). Warly et al. (1992) suggested that in sheep the nutritional limitations of rice straw could be overcome by supplementation with SBM to provide an optimum ruminal condition for rumen microorganisms. However, until now, information about the feeding effects of rice straw supplemented with SBM (RSS-diet) on metabolism of VFA, the important metabolites in ruminants, is not available.

Acetate is one of the major VFA which fulfills 50% energy requirement in sheep (Skutches et al., 1979). Production of this acid in the rumen largely depends upon the quantity of dietary feed intake, type of dietary carbohydrate and time course of feeding (Van Nevel and Demeyer, 1988; Bergman, 1990; Sutton et al., 2003). Therefore, it

could be expected that acetate metabolism will be influenced in sheep fed RSS-diet because it provides required NH_3 for increasing microbial activity (Warly et al., 1992). The current study was designed to evaluate the effects of RSS-diet on kinetics of plasma acetate turnover rate using a [1-^{13}C]Na acetate isotope dilution technique as well as rumen fermentation characteristics and blood metabolites concentration in sheep.

Materials and Methods

Sheep, diets and management

The study used 4 sound healthy crossbred (Corriedale x Suffolk) shorn sheep of both sexes, averaging 34.3±5.7 kg of BW at the beginning of the study. Animal care procedures and protocol were the same as before (**Chapter1**). The sheep were assigned to two dietary treatments including either a RSS-diet or a MH-diet with 100% ME and 120% CP for maintenance. Chemical compositions of experimental diets were shown in **Table 2.1**. The ME was estimated 1.30 kcal/g for rice straw, 2.77 kcal/g for SBM and 1.73 kcal/g for mixed hay according to same feeding standard as mentioned in **Chapter1**. Feed allowance was rice straw 59.4 g/kg$^{0.75}$/d supplemented with SBM 7.4 g/kg$^{0.75}$/d for the RSS-diet and mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet. The experiment was performed using a crossover design with two 24 d periods. Two sheep were fed the RSS-diet during the first period and then the MH-diet during the second period, and the other two sheep were fed in the reverse order. The sheep were housed in individual pens in an animal barn during the first 17 d of each dietary period. On d 18 of each dietary treatment, sheep were moved to individual metabolic cages in a controlled environment chamber at an air temperature of 23±1 °C with lighting from 08:00 h to

22:00 h. The animals were fed once daily at 14:00 h and the manger was untouched till next morning. Water was available *ad libitum*. The sheep were weighed on d 1, 8, 18 and 24 of each dietary treatment. The experimental layout is shown in **Figure 2.1**.

Nitrogen balance

A five d (from d 19 to d 23) long N balance trial was carried out for each dietary treatment. Procedures of urine and feces samples were treated as described before in **Chapter1**.

Table 2.1. Chemical composition of experimental diet on air dry matter basis

Items (%)	MH	RS	SBM
Dry matter	89.2	93.3	89.0
Crude protein	11.2	5.1	45.2
Crude ash	11.1	15.9	6.2
Crude fiber	29.3	33.8	4.2
NDF	69.4	72.3	8.1
ADF	38.1	44.4	6.1
ADL	3.9	2.0	0.1

MH = Mixed hay of orchardgrass and reed canarygrass, RS = Rice straw,
SBM = Soybean meal, NDF = Neutral detergent fiber,
ADF = Acid detergent fiber,
ADL = Acid detergent lignin.

Collection of rumen fluid

On d 23 of each dietary treatment, rumen fluid was collected 2 h after feeding through the stomach tube inserted orally. The pH value of the rumen fluid was measured by a pH meter immediately after collection. A sub-sample was centrifuged and then an aliquot of supernatant was acidified for measuring rumen NH_3 concentration

as described in **Chapter1**. The acidified supernatant and residuals were stored at -30 °C for further analysis.

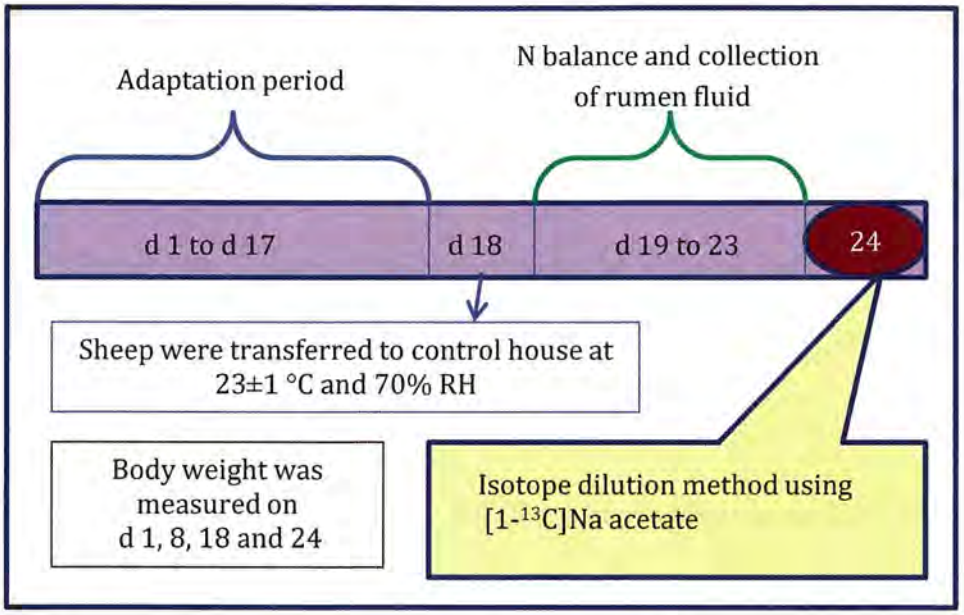

Figure 2.1. A schematic diagram of experimental layout showing sampling protocols

Isotope dilution method

An isotope dilution method using [1-^{13}C]Na acetate was carried out to determine TR of plasma acetate on d 24 of each dietary period. Isotope dilution procedure, rate of isotope infusion, method of infusion and blood sampling procedures were same as described in **Chapter1**. After the end of the experiment plasma was separated from blood cells by centrifuging as before (**Chapter1**) and supernatant was then stored at -30 °C for later analysis.

Chemical analysis

Chemical analyses of N balance and dietary composition were the same as described in **Chapter1**. Rumen VFA concentrations in rumen fluid were determined

using the GC after steam distillation, and rumen NH_3 concentration was measured using the colorimetric method as described previously (**Chapter1**). Plasma free AA, NH_3 and urea concentrations were determined using the automated AA analyzer. Concentration of plasma glucose and NEFA were determined enzymatically using the diagnostic kit as described previously (**Chapter1**). Plasma [1-^{13}C]acetate enrichment and concentrations of plasma VFA and lactate were determined using the selected ion monitoring system with the GC/MS as described in **Chapter1**.

Calculation

Mean values with SEM are given. For the isotope dilution method, the plasma acetate TR was calculated using the equation given by Tserng and Kalhan (1983) as follows:

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

Where I is the infusion rate of [1-^{13}C]Na acetate and E is the plasma isotopic enrichment of [1-^{13}C]acetate at steady state.

Statistical analysis

All data were statistically analyzed with the MIXED procedure of SAS (1996) with diet and period as the fixed effect and sheep as the random effect. Results were considered significant at the $P < 0.05$ level and a tendency was defined as $0.05 \leq P < 0.10$.

Results

Daily profile and N balance

Body weight gain did not differ between diets (**Table 2.2**). Dry matter intake was higher ($P = 0.01$) for the RSS-diet and estimated ME intake was similar between dietary treatments. Nitrogen intake did not differ between diets. Nitrogen excretion through feces was lower ($P = 0.01$) for the RSS-diet, but N excretion through urine and N retention did not differ between diets. The reduction of N excretion through feces resulted in considerably higher ($P = 0.004$) N digestibility for the RSS-diet compared to the MH-diet.

Table 2.2. Dietary effects on body weight (BW) gain, dry matter (DM) intake, estimated metabolizable energy (ME) intake, nitrogen (N) balance and N digestibility in sheep

Items	MH-diet	RSS-diet	SEM	*P*- value
No. of sheep	4	4		
BW gain (g/d)	57	30	30	0.40
DM intake (g/kg$^{0.75}$/d)	50	58	2	0.01
ME intake (kcal/kg$^{0.75}$/d)	100	98	1	0.43
N intake (g/kg$^{0.75}$/d)	0.99	0.93	0.02	0.20
N in feces (g/kg$^{0.75}$/d)	0.38	0.26	0.03	0.01
N in urine (g/kg$^{0.75}$/d)	0.39	0.39	0.02	0.99
N balance (g/kg$^{0.75}$/d)	0.21	0.28	0.03	0.25
N digestibility (%)	62	73	2	0.004

MH = Mixed hay of orchardgrass and reed canarygrass, RSS = Rice straw supplemented with soybean meal, SEM = Standard error of the mean.

Rumen fermentation characteristics

Rumen fermentation parameters for each treatment are presented in **Table 2.3.** Rumen pH was similar between the dietary treatments. Concentration of rumen NH_3

tended to be higher (P = 0.07) for the RSS-diet than the MH-diet. Concentrations of rumen total VFA and acetate did not differ between diets, and those of other individual VFA were higher (P < 0.05) for the RSS-diet compared to the MH-diet.

Table 2.3. Dietary effects on rumen pH, concentrations of rumen ammonia (NH_3) and volatile fatty acids (VFA) in sheep

Items	MH-diet	RSS-diet	SEM	*P*-value
No. of sheep	4	4		
pH	6.90	6.93	0.07	0.80
(mmol/L)				
NH_3	4.14	6.25	0.76	0.07
Total VFA	77.0	93.0	4.8	0.11
Acetate	58.0	66.0	3.1	0.26
Propionate	12.0	17.0	1.2	0.02
Iso-Butyrate	0.8	1.1	0.1	0.01
Butyrate	4.3	6.6	0.5	0.04
Iso-Valerate	0.8	1.5	0.1	0.01
Valerate	0.5	0.8	0.1	0.02

MH = Mixed hay of orchardgrass and reed canarygrass, RSS = Rice straw supplemented with soybean meal, SEM = Standard error of the mean.

Plasma metabolites

Plasma total free AA concentrations tended to be lower (P = 0.08) for the RSS-diet compared to the MH-diet (**Table 2.4**). Among the individual plasma free AA, lysine was higher (P = 0.02) and proline was lower (P = 0.03) for the RSS-diet compared to the MH-diet, and those of other individual AA did not differ significantly between diets. Plasma NH_3 concentration was lower (P = 0.02) and plasma urea concentration tended to be higher (P = 0.08) for the RSS-diet than the MH-diet. Concentrations of plasma NEFA, glucose and lactate were not affected by dietary treatments.

Table 2.4. Dietary effects on plasma metabolite concentrations in sheep

Items	MH-diet	RSS-diet	SEM	*P*-value
No. of sheep	4	4		
Essential AA (μmol/L)				
Threonine	287	146	43	0.12
Valine	248	177	40	0.05
Methionine	22	11	7	0.32
Iso-leucine	115	78	15	0.07
Leucine	157	96	23	0.12
Phenylalanine	75	47	12	0.15
Histidine	25	22	3	0.34
Lysine	24	36	4	0.02
Nonessential AA (μmol/L)				
Aspartic acid	16	14	2	0.17
Serine	178	123	36	0.07
Asparagine	89	66	12	0.07
Glutamic acid	213	152	23	0.05
Glutamine	116	154	25	0.07
Glycine	636	513	93	0.06
Alanine	228	206	19	0.38
Tyrosine	67	42	10	0.10
Tryptophan	137	150	18	0.34
Arginine	144	129	13	0.49
Proline	118	45	35	0.03
Total AA (μmol/L)	2895	2208	322	0.08
NH_3 (μmol/L)	398	310	34	0.02
Urea (mmol/L)	5.04	6.80	0.65	0.08
Glucose (mmol/L)	3.83	3.59	0.12	0.12
NEFA (μEq/L)	339	472	11	0.10
Lactate (μmol/L)	357	405	40	0.12

MH = Mixed hay of orchardgrass and reed canarygrass, RSS = Rice straw supplemented with soybean meal, SEM = Standard error of the mean, AA = Amino acid, NEFA = Non-esterified fatty acid.

Plasma acetate kinetics

Plasma acetate concentration and [1-^{13}C]acetate enrichment remained constant during the latter half of the [1-^{13}C]Na acetate infusion (**Figure 2.2**). Plasma acetate

concentration determined during the last 2 h continuous infusion of the isotope dilution did not differ between diets (**Table 2.5**). Turnover rate of plasma acetate determined from the enrichment of plasma [1-^{13}C]acetate did not differ between dietary treatments.

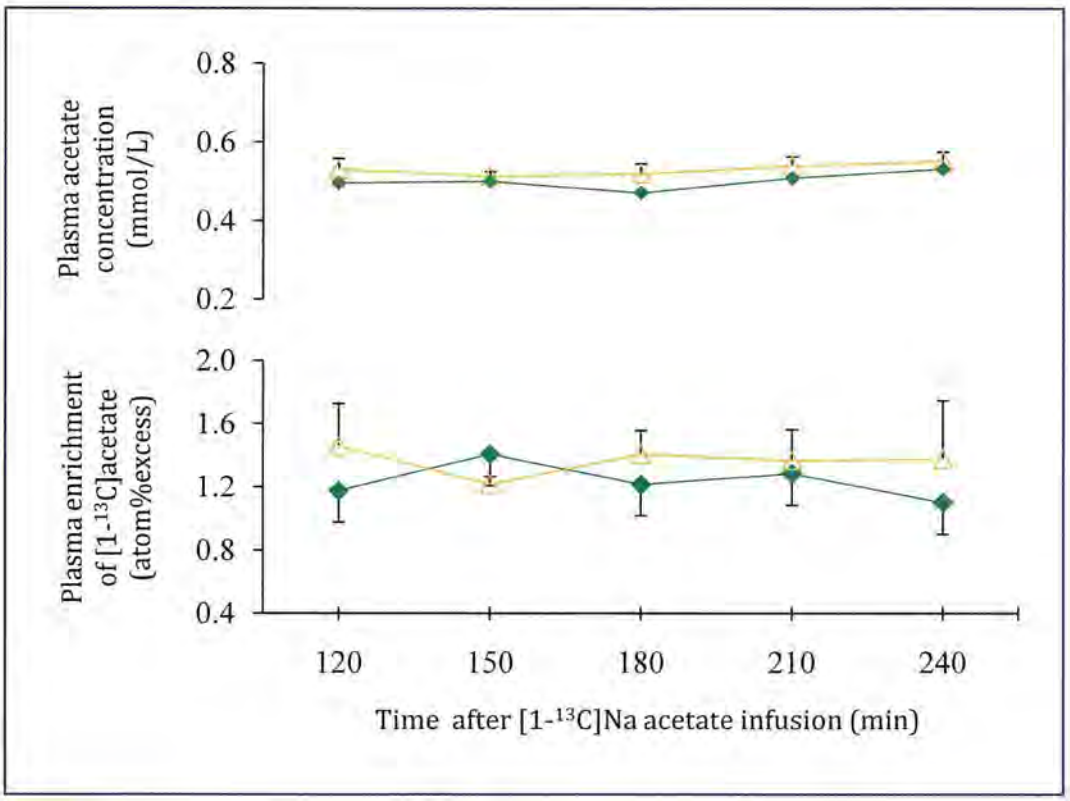

Figure 2.2. Time course changes in plasma acetate concentration and [1-^{13}C]acetate enrichment during the latter half of the primed-continuous infusion of [1-^{13}C]Na acetate in sheep fed the RSS-diet (△) and the MH-diet (♦). (Means ± SEM).

Table 2.5. Dietary effects on kinetics of plasma acetate concentration and turnover rate (TR) in sheep

Items	MH-diet	RSS-diet	SEM	*P*-value
No. of sheep	4	4		
Plasma acetate				
Concentration (μmol/L)	511	541	20	0.31
TR (mmol/kg$^{0.75}$/h)	5.18	4.57	0.34	0.15

MH = Mixed hay of orchardgrass and reed canarygrass, RSS = Rice straw supplemented with soybean meal, SEM = Standard error of the mean.

Discussion

The present experiment demonstrated that rice straw supplemented with a high energy protein source showed comparable performance to mixed hay on plasma acetate metabolism, and improved performance over mixed hay on rumen VFA, NH_3 and N digestibility in sheep.

Daily profile and N balance

In the present experiment both diets were consumed by the sheep. For both diets animals were in positive BW gain. It was indicated the similar palatability for both diets and sheep were free from metabolic disorder throughout the experimental period. In the current study considerably higher N digestibility for the RSS-diet might be due to presence of rumen degradable protein source which could rapidly degrade the dietary nitrogenous substrates in the rumen. In previous studies it was shown that inclusion protein source to low quality roughage diets made N available for microbial growth and increased the N digestibility in ruminants (Pradhan et al., 1996; Nguyen et al., 2008). Comparing with the results of the previous study (**Chapter1**), it is likely that dietary N intake and N digestibility were enhanced in sheep fed rice straw supplemented with SBM.

Rumen fermentation characteristics

Rumen fermentation characteristics such as NH_3 and VFA concentrations were influenced in sheep fed rice straw supplemented with SBM. It was indicated that SBM supplementation to rice straw made a better rumen environment for dietary carbohydrate fermentation providing easily fermentable nitrogenous and energy

substrate. The present results were supported by Warly et al. (1992), who observed that SBM supplementation to rice straw increased the rumen VFA concentrations by influencing the dietary organic matter fermentation in the rumen. The higher propionate concentration in the rumen for the RSS-diet is in accordance with the previous findings of Kirkpatrick and Kennelly (1989), who revealed that supplementation of SBM with chopped brome-alfalfa hay and barley increased rumen propionate concentration in heifers. Similar rumen pH for both diets in the present study indicated the balance of rumen VFA and NH_3 between the diets. A similar trend was found in sheep fed rice straw supplemented with SBM as protein source (Nguyen et al., 2008).

Plasma metabolites

Several factors are responsible to influence the level of AA in blood such as absorption, tissue utilization and liver catabolism, dietary types and frequency of feeding as described previously (**Chapter1**). The present results revealed that SBM supplementation to rice straw did not influence the plasma total free amino acid concentrations determined at 22 h after feeding. The effect of SBM supplementation would not be reflected in plasma free amino acids due to its rapid degradation in the rumen. This is in agreement with the previous findings of Santos et al. (1984). The lower supply of N from rice straw resulted in a tendency of lower plasma total free AA in this study. In the previous study (**Chapter1**) plasma free AA were considerably lower in sheep fed rice straw only due to its lower N content. A tendency of higher urea concentration in plasma for the RSS-diet reflected the rapid absorption of NH_3 from rumen and then converted into urea in the liver in accordance with Milano and Lobley

(2001). The present findings were also agreed with the observation of Sano and Shibasaki (2011), who reported that N source supplementation influenced plasma urea concentration in sheep fed concentrate-based diets. Plasma NEFA concentration showed variation related primarily to the time of feeding (Bowden, 1971; Udum et al., 2008). Higher plasma NEFA concentration might be related to a prolonged sampling time relative to feeding in the current study. This is in accordance with Russel et al. (1967), who found that NEFA levels in ewes increased 3 to 5 times, from 2 to 3 h after feeding to 24 h after feeding because of addipose tissue mobilization. Plasma NEFA is an important indicator of nutritional status in animal, and thus the present results indicated the similar nutritional status between the diets in sheep. Moreover, the similar plasma lactate concentration between the diets indicated the overall efficiency of energy utilization for both diets in accordance with the findings of Gill et al. (1986).

Plasma acetate kinetics

Until now, the feeding effects of rice straw supplemented with SBM on plasma acetate kinetics have not been investigated in sheep. Acetate is the primary fatty acid produced in the rumen, absorbed through rumen wall and then to the portal vein, thus plasma acetate TR depends largely on acetate production in the rumen as described in **Chapter1**. In the present study, rice straw supplemented with SBM did not influence the plasma acetate TR in accordance with Reynolds et al. (1994), who reported that acetate absorption by the portal-drained viscera was not influenced in heifers fed concentrate diet. Moreover, Pethick and Lindsay (1982) also reported that plasma acetate concentration as well as plasma acetate TR were unaffected by concentrate

supplementation to diet in lactating sheep. The similar plasma acetate TR in the current study might be partially due to give the feed as iso-caloric and iso-nitrogenous, even though the different diets were used. The numerical values of plasma acetate TR of the present study were close to the data reported in sheep using the same isotope dilution technique (Al-Mamun et al., 2011). Moreover, the numerical values of plasma acetate TR of the present study slightly differed from the data of my previous study in sheep fed rice straw only (**Chapter1**). This may be due to the differences of feeding level, feeding frequency and sampling time relative to feeding. From the present study it was found that SBM supplementation only to rice straw was not sufficient to influence intermediary metabolism in sheep. Thus further research should be done to evaluate the feeding effects of rice straw supplemented with sources of protein and soluble carbohydrate on intermediary metabolism of plasma nutrients in sheep.

Chapter3

Intermediary Metabolism of Plasma Glucose and Leucine Kinetics in Sheep Fed Rice Straw Supplemented with Urea and Molasses

Introduction

The previous study (**Chapter2**) demonstrated that SBM supplementation to rice straw as a high energy protien source did not influence the intermediary metabolism of plasma nutrients in sheep. Intermediary metabolism of plasma nutrient is correlated to microbial activities in the rumen. For increasing the microbial activities it is necessary to supply nitrogenous substrates along with soluble carbohydrate source in ruminants fed rice straw, because soluble carbohydrates influenced the microbial protein synthesis in the rumen of cattle (Rooke and Armstrong, 1989). An easily available soluble carbohydrate source is molasses, which supplies the required energy for microbial activities (Toppo et al., 1997). Supplementation of nitrogenous substrates to rice straw is the way to recover its CP deficiency and to improve its digestibility as mentioned in **Chapter2**. Urea is the most common source of supplemental nitrogenous substrate to provide NH_3 for rumen microbial growth (Tedeschi et al., 2002; Zinn et al., 2003). It was found that the supplementation of urea in combination with molasses to straw diets improved feed intake, digestive function along with ruminal characteristics in ruminant (Can et al., 2004; Wu et al., 2005; Hue et al., 2008).

It can be expected that rice straw supplemented with urea and molasses (RSUM-diet) will influence nutrient metabolism as well as rumen fermentation characteristics and N balance in sheep through providing required NH_3 and energy for rumen microbial activities to increase the dietary carbohydrate fermentation. Therefore, the present study was designed to evaluate the effect of a RSUM-diet on turnover rates of plasma glucose and Leu along with the determination of the N balance, rumen fermentable characteristics and plasma metabolite concentrations in sheep compared with a MH-diet.

Materials and Methods

Animals, diets and managements

Experimental procedures including animal care, cannulation and blood sampling were reviewed and approved as before (**Chapter1**). Four sound healthy crossbred (Corriedale x Suffolk) shorn sheep, weighing 46.6±2.2 kg of BW were used. Two dietary treatments were tested; one was a RSUM-diet, the other was a MH-diet using 100% ME for maintenance. Chemical composition of experimental feed is shown in **Table 3.1**. The ME was estimated at 1.30 kcal/g for rice straw, 2.62 kcal/g for molasses and 1.73 kcal/g for mixed hay, according to the same feeding standard as mentioned before (**Chapter1**). Feed allowance was rice straw 59.7 g/kg$^{0.75}$/d supplemented with urea 0.84 g/kg$^{0.75}$/d and molasses 7.6 g/kg$^{0.75}$/d for the RSUM-diet, and mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet. Urea and molasses were given on chopped (3-4 cm) rice straw immediately before feeding. Feed was given at 8:00 h and 20:00 h and fresh drinking water was available *ad libitum*.

Table 3.1. Chemical composition of experimental feed on air dry matter basis

Items (%)	Mixed hay	Rice straw	Molasses	Urea
Dry matter	93.3	94.7	84.6	ND
Crude protein	12.0	4.6	12.4	288
Crude ash	10.8	14.4	12.5	ND
Crude fiber	28.6	31.9	8.6	ND
NDF	68.8	73.1	30.0	ND
ADF	32.7	41.7	20.6	ND
ADL	2.0	2.4	5.4	ND

NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, ND = Not determined.

The experiment was performed using a crossover design with two 21 d period. Two sheep were fed the MH-diet during the first period and then the RSUM-diet during the second period, and the other two sheep were fed in the reverse order. The sheep were housed in individual pens in an animal barn during the adjustment period (first two weeks), and on d 15, the sheep were moved to the controlled house at an air temperature of 23±1 °C with lighting from 8:00 h to 22:00 h and maintained in wooden metabolism stalls designed for total collection of feces and urine. The sheep were weighed on day of starting the experiment and every 7 d intervals of each dietary period. All exprimental procedures were carried out without noticeable stress to the animals. Timetable of the experiment is shown in **Figure 3.1.**

Nitrogen balance

A five d long (from d 16 to d 20) N balance trial was conducted for each dietary period. Procedures of urine, feces and feed refusal samples were treated as described in **Chapter1.**

Collection of rumen fluid

Rumen fluid was collected from each sheep 2 h after the morning feeding with an orally inserted stomach tube on d 20 of each dietary period. The pH value was measured by the pH meter immediately after collection of the rumen fluid. A sub-sample was centrifuged and then an aliquot of supernatant was acidified by 0.1 N HCl for measuring rumen NH_3 concentration as described in **Chapter1**. The acidified supernatant and residuals were stored at -30 °C for further analysis.

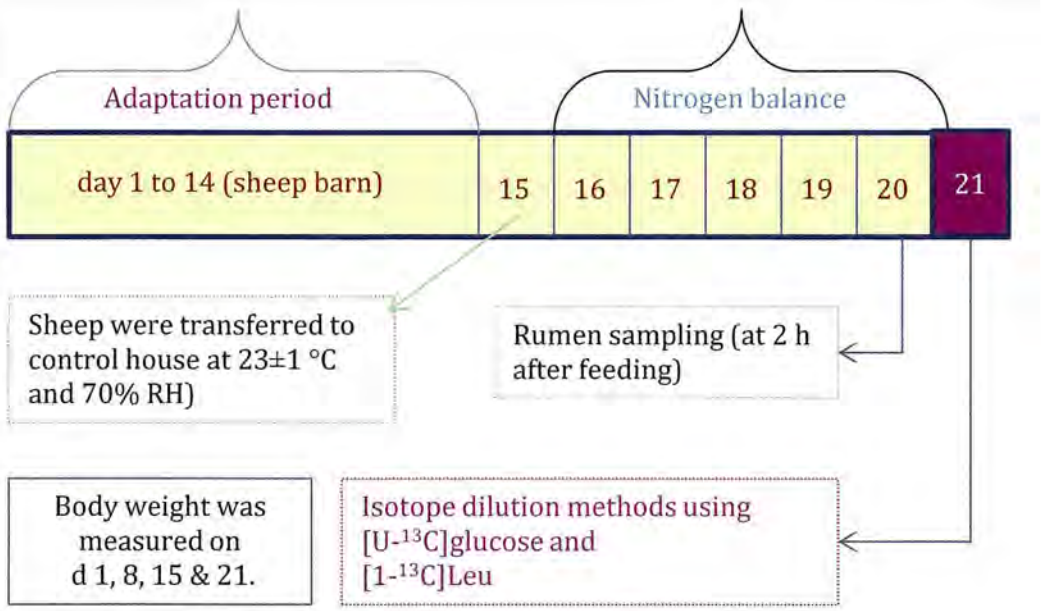

Figure 3.1. Schematic diagram of experimental layout showing the sampling protocols

Isotope dilution method

Isotope dilution methods using [U-^{13}C]glucose and [1-^{13}C]Leu were conducted to determine the TR of plasma glucose and Leu 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 each isotope dilution method. The isotope dilution methods, rate of infusion, method of infusion and blood sampling procedures were the same as described in **Chapter1**. After end of the experiment plasma was separated from blood cells by centrifuging as described before (**Chapter1**) and supernatant was then stored at -30 °C for later analysis.

Chemical analysis

Chemical analyses of N balance and dietary composition were the same as described in **Chapter1**. Rumen VFA concentrations in rumen fluid were determined using the GC after steam distillation, and rumen NH_3 concentration was measured using the colorimetric method as described previously (**Chapter1**). Plasma free AA, NH_3, urea and NEFA concentrations were determined as described previously (**Chapter1**). Plasma enrichments of [U-^{13}C]glucose were determined using the selected ion monitoring system with the GC/MS. Concentration of plasma glucose was determined using the method described in **Chapter1**. Plasma AA and α-keto acids were separated and converted to MTBSTFA derivatives according to the procedures described in **Chapter1.** Isotopic enrichment of plasma [1-^{13}C]Leu and concentration of plasma Leu were measured by the selected ion monitoring system using the GC/MS.

Calculation

Results are presented as mean values with SEM. Turnover rates of plasma glucose and Leu were calculated using the equation described by Tserng and Kalhan (1983) as follows:

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

Where, I is the infusion rate of [U-^{13}C]glucose and [1-^{13}C]Leu isotope and E is the plasma isotopic enrichment of [U-^{13}C]glucose and [1-^{13}C]Leu during the steady state.

The WBPS and WBPD were determined from the relationship among WBPF, N absorption and urinary N excretion using same equation described in **Chapter1** as follows:

WBPF = LeuTR/0.066

WBPS = WBPF- (urinary N × 6.25)

WBPD = WBPF- (absorbed N × 6.25)

Statistical analysis

All data were statistically analyzed using the MIXED procedure of SAS (1996). The least square means statement was used to test the effects of period and diet. Results were considered significant at the $P < 0.05$ level and a tendency was defined as $0.05 \leq P < 0.10$.

Results

Daily profile and N balance

The BW gain did not differ between diets (**Table 3.2**). Dry matter intake was greater ($P = 0.02$) in sheep fed the RSUM-diet than the MH-diet. Estimated ME intake did not differ between diets. Nitrogen intake and N excretion through feces were lower ($P < 0.05$) for the RSUM-diet than the MH-diet, and N digestibility did not differ between diets. Nitrogen excretion through urine did not differ between diets and N retention was lower ($P = 0.03$) for the RSUM-diet compared to the MH-diet.

Table 3.2. Dietary effects on body weight (BW) gain, dry matter (DM) intake, estimated metabolizable energy (ME) intake, nitrogen (N) balance and N digestibility in sheep

Items	MH-diet	RSUM-diet	SEM	*P*-value
No. of sheep	4	4		
BW gain (g/d)	89	30	48	0.26
DM intake (g/kg$^{0.75}$/d)	54	59	2	0.02
ME intake (kcal/kg$^{0.75}$/d)	99	92	2	0.21
N intake (g/kg$^{0.75}$/d)	1.10	0.94	0.05	0.01
N in feces (g/kg$^{0.75}$/d)	0.37	0.30	0.02	0.01
N in urine (g/kg$^{0.75}$/d)	0.45	0.46	0.01	0.90
N retention (g/kg$^{0.75}$/d)	0.28	0.18	0.04	0.03
N digestibility (%)	67	67	1	0.57

MH = Mixed hay of orchardgrass and reed canarygrass, RSUM = Rice straw supplemented with urea and molasses, SEM = Standard error of the mean.

Rumen fermentation characteristics

Rumen pH did not differ between dietary treatments (**Table 3.3**). Concentration of rumen NH_3 was higher ($P = 0.03$) for the RSUM-diet than the MH-diet. Concentrations of rumen total VFA tended to be higher ($P = 0.09$) for the RSUM-diet than the MH-diet. Acetate concentration in the rumen did not differ between diets. Concentration of propionate in the rumen was higher ($P = 0.01$) for the RSUM-diet than the MH-diet. Concentration of iso-butyrate was lower ($P = 0.01$) and iso-valerate tended to be lower ($P = 0.05$) for the RSUM-diet compared to the MH-diet, but concentrations of butyrate and valerate in the rumen did not differ between diets.

Plasma metabolites

Plasma free AA determined at pre-infusion of isotope dilution did not differ significantly between diets, except that lysine, glutamic acid and glutamine were higher ($P < 0.05$) for the RSUM-diet compared to the MH-diet (**Table 3.4**). Concentration of plasma NH_3 tended to be higher ($P = 0.07$) for the RSUM-diet and urea did not differ

between diets. Concentration of plasma NEFA was lower ($P = 0.03$) for the RSUM-diet compared to the MH-diet.

Table 3.3. Dietary effects on rumen pH, concentrations of rumen ammonia (NH_3) and volatile fatty acids (VFA) in sheep

Items	MH-diet	RSUM-diet	SEM	*P*-value
No. of sheep	4	4		
pH	6.82	6.86	0.10	0.81
(mmol/L)				
NH_3	4.56	7.06	1.06	0.03
Total VFA	79.5	88.4	6.4	0.09
Acetate	59.5	61.3	4.4	0.38
Propionate	13.7	20.9	2.6	0.01
Iso-Butyrate	0.7	0.3	0.1	0.01
Butyrate	4.6	5.3	0.5	0.17
Iso-Valerate	0.7	0.3	0.2	0.05
Valerate	0.5	0.3	0.1	0.34

MH = Mixed hay of orchardgrass and reed canarygrass, RSUM = Rice straw supplemented with urea and molasses, SEM = Standard error of the mean.

Plasma glucose and Leu kinetics

Plasma glucose concentration and [U-^{13}C]glucose enrichment remained constant during latter half of the isotope infusion (**Figure 3.2**). Concentrations of plasma glucose determined during the latter half of the primed-cotinuous infusion of isotope dilution method did not differ between the diets (**Table 3.5**). Turnover rate of plasma glucose calculated from the enrichment of plasma [U-^{13}C]glucose did not differ between diets.

Table 3.4. Dietary effects on plasma metabolite concentrations in sheep

Items	MH-diet	RSUM-diet	SEM	*P*-value
No. of sheep	4	4		
Essential AA (μmol/L)				
Threonine	194	163	41	0.07
Valine	228	185	34	0.13
Methionine	37	29	14	0.49
Iso-leucine	92	74	16	0.26
Leucine	100	85	20	0.38
Phenylalanine	45	41	6	0.48
Histidine	24	23	2	0.43
Lysine	32	43	5	0.04
Nonessential AA (μmol/L)				
Aspartic acid	14	12	2	0.11
Serine	205	176	14	0.33
Asparagine	66	70	11	0.93
Glutamic acid	253	332	24	0.02
Glutamine	84	121	21	0.01
Glycine	610	583	44	0.11
Alanine	183	197	18	0.09
Tyrosine	58	63	11	0.39
Tryptophan	149	136	25	0.41
Arginine	131	101	15	0.06
Proline	67	68	12	0.78
Total AA (μmol/L)	2437	2565	162	0.24
NH_3 (μmol/L)	383	419	16	0.07
Urea (mmol/L)	7.83	8.37	0.89	0.18
NEFA (μEq/L)	284	148	62	0.03

MH = Mixed hay of orchardgrass and reed canarygrass, RSUM = Rice straw supplemented with urea and molasses, SEM = Standard error of the mean, AA = Amino acid, NEFA = Non-esterified fatty acid.

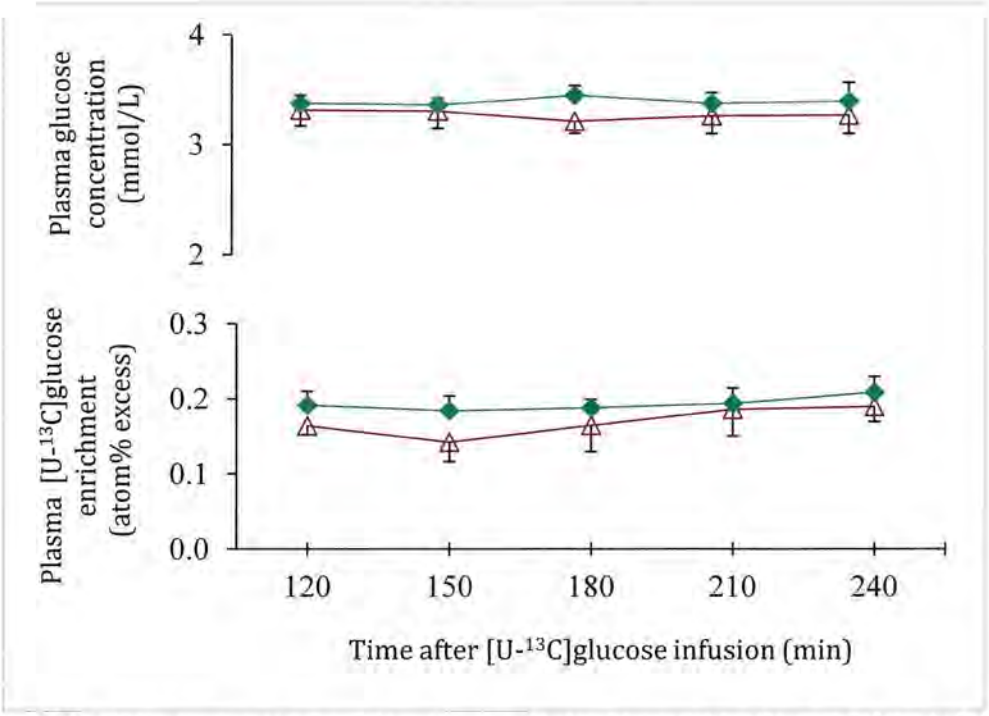

Figure 3.2. Time course changes in plasma glucose concentration and [U- ^{13}C]glucose enrichment during the last 2 h continuous infusion of [U-^{13}C]glucose in sheep fed the RSUM-diet (Δ) and the MH-diet (♦). (Means ± SEM).

Plasma Leu concentration and [1-^{13}C]Leu enrichment were stable during the latter half of isotope infusion (**Figure 3.3**). Concentration of plasma Leu determined during the latter half of the primed-continuous infusion of isotope dilution did not differ between dietary treatments (**Table 3.5**) Turnover rate of plasma Leu as well as WBPS and WBPD determined from plasma [1-^{13}C]Leu enrichment did not differ between the RSUM-diet and the MH-diet.

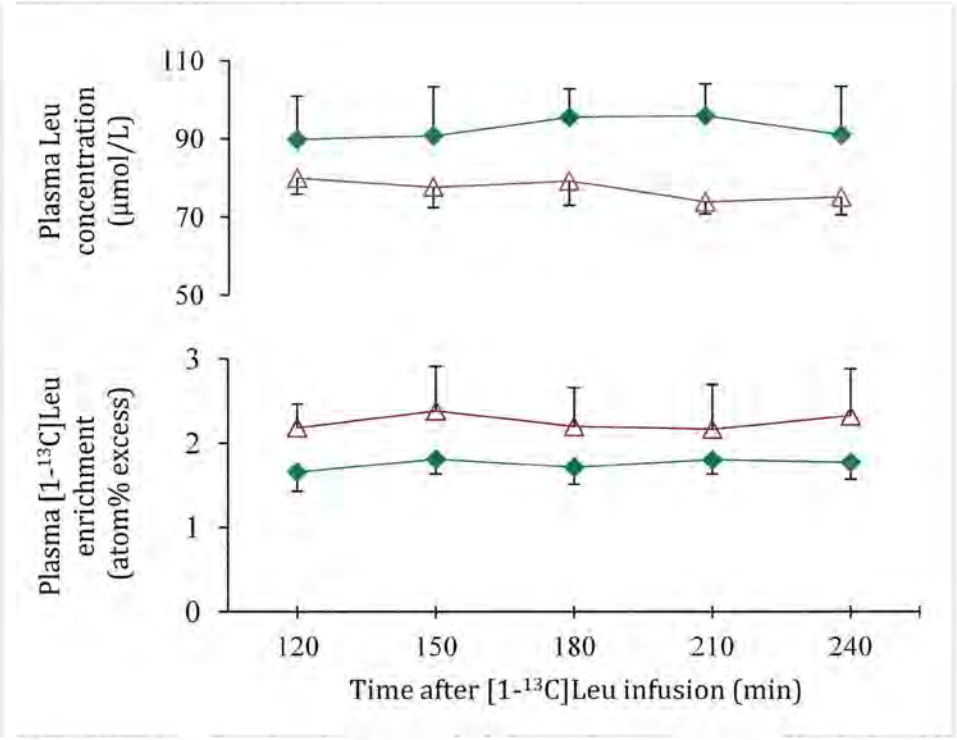

Figure 3.3. Time course changes in plasma Leu concentration and [1-^{13}C]Leu enrichment during the last 2 h continuous infusion of [1-^{13}C]Leu in sheep fed the RSUM-diet (Δ) and the MH-diet (♦). (Means ± SEM).

Table 3.5. Dietary effects on kinetics of plasma glucose and leucine (Leu) metabolism in sheep

Items	MH-diet	RSUM-diet	SEM	*P*-value
No. of sheep	4	4		
Plasma glucose				
Concentration (mmol/L)	3.38	3.28	0.11	0.51
TR (mmol/kg$^{0.75}$/h)	1.43	1.52	0.18	0.31
Plasma Leu				
Concentration (μmol/L)	93.4	77.3	6.6	0.11
TR (μmol/kg$^{0.75}$/h)	285	272	49	0.76
WBPS (g/kg$^{0.75}$/d)	10.8	10.0	2.3	0.68
WBPD (g/kg$^{0.75}$/d)	9.0	9.1	2.4	0.98

MH = Mixed hay of orchardgrass and reed canarygrass, RSUM = Rice straw supplemented with urea and molasses, SEM = Standard error of the mean, TR = Turnover rate, WBPS = Whole body protein synthesis, WBPD = Whole body protein degradation.

Discussion

The present study demonstrated that untreated rice straw supplemented with urea and molasses showed improved performance over mixed hay on rumen fermentation characteristics, and comparable performance to mixed hay on turnover rates of plasma glucose and Leu as well as WBPS and WBPD in sheep.

Daily profiles and N balance

The animals were in positive BW gain for both diets. No significant difference was found between diets in relation to daily BW gain. A similar trend was found in lambs fed urea treated rice straw supplemented with molasses (Hue et al., 2008). Greater DM intake for the RSUM-diet in the present study might be due to availability of urea and molasses, which lead to progressive change in rumen fermentation. The numerical values of DM intake in the present study were comparable with the data reported by Singh et al. (1995). Although feed was given as iso-nitrogenous, lower N intake for the RSUM-diet might be due to loss of some N through residue of rice straw. No significant diffrence occured in N digestibility between diets in the present study. This might be due to rapid hydrolysis of urea in the rumen. Can et al. (2004) reported the improved N digestibility in sheep fed wheat straw supplemented with urea and molasses than only wheat straw. Similarly comparing with the results of **Chapter1,** it can be noted that N intake as well as N digestibility were improved in sheep fed rice straw supplemented with urea and molasses than rice straw only.

Rumen fermentation characteristics

Rumen pH determined at 2 h after feeding was within the normal range for both diets. Similar rumen pH between diets was an indication of balance between the concentrations of VFA and NH_3 in the rumen as described previously in **Chapter2**. The numerical values of rumen pH of the present study were comparable with the data reported in sheep fed urea supplemented diet (Sano et al., 2009). Leng (1990) reported that the critical level of NH_3 is between 2.9 and 14.7 mmol/L of rumen liquor for promoting the rumen fermentation rate. In the present study rumen NH_3 concentration was within the normal range for promoting the rumen fermentation. The higher NH_3 concentration in the rumen for the RSUM-diet was likely due to the presence of urea in supplements. This is in accordance with Jain et al. (2005), who reported that in goat kids the higher solubility of urea and its rapid hydrolysis by rumen microorganisms increased the NH_3 concentration in the rumen. Supplementation of urea and molasses to low quality roughage diets made better rumen environment for dietary carbohydrate fermentation through supplying adequate NH_3 and energy for rumen microbial growth (Srinivas and Gupta, 1997). A tendency of higher rumen total VFA for the RSUM-diet in the present study indicated the well fermentation of dietary carbohydrate in the rumen. Similarly Jain et al. (2005) observed that rumen VFA concentrations were affected by urea, molasses and mineral granules supplementation with rice straw in goat kids. Propionate concentration in the rumen is generally affected by the readily fermentable carbohydrate in diets (Van Houtert, 1993). Higher concentration of ruminal propionate for the RSUM-diet than the MH-diet was due to presence of molasses as a source of

readily fermentable carbohydrate. The present results were supported by Broderick and Radloff (2004), who mentioned that molasses supplementation to diets influenced the propionate concentration in the rumen.

Plasma metabolites

Plasma free AA concentrations in peripheral blood are related to the quantity of dietary protein that reach the small intestine (Bergen et al., 1973). Pendlum et al. (1976) suggested that changes in plasma free AA patterns in ruminants were associated with the degree of protein degradation and synthesis in the rumen. Elevated plasma AA for the RSUM-diet in the present study might be due to adequate supply of NH_3 and easily fermentable energy substrates for microbial protein synthesis. Concentration of plasma NH_3 is positively associated with the production of NH_3 in the rumen as described in **Chapter2.** A tendency of higher plasma NH_3 concentarion for the RSUM-diet might be due to rapid absorption of NH_3 from the rumen. The present result is in accordance with Sano et al. (2009), who suggested that when urea was supplemented to the basal diet, the postprandial plasma NH_3 increased markedly because a large part of the NH_3 produced from the supplemental urea in the rumen and directly absorbed into portal blood. Plsama NEFA concentration is the indicator of energy status in ruminants (Fox et al., 1991). Because, NEFA is released into blood when adipose tissues are mobilized to supply the metabolic needs of animal, primarily the need of energy. In the current study, lower NEFA concentration for the RSUM-diet is likely due to the slower consumption of the RSUM-diet that made nutrients available to reduce the body lipid mobilization.

Lower plasma NEFA concentration for the RSUM-diet indicated its improved nutritional status due to nitrogenous substrate and soluble carbohydrate supplementation.

Plasma glucose and Leu kinetics

Until now, plasma glucose metabolism was not observed in sheep fed rice straw supplemented with urea and molasses. Whole body glucose TR is influenced by endocrine hormones (Sano et al., 1996). Other studies reported that rates of plasma glucose turnover were associated with the dietary intake level and supply of gluconeogenic substrates to the liver as described previously (**Chapter1**). In previous studies it was suggested that the precursor availability is an important factor in regulating gluconeogenesis (Schmidt and Keith, 1983; Oba and Allen, 2003). Plasma glucose TR in the present study did not differ between diets, although the rumen propionate, a major glucose precursor, was higher for the RSUM-diet. The present results were supported by Seal and Parker (1994), who reported that increasing supply of glucogenic propionate did not influence the plasma glucose TR in steers. Although the isotope dilution method was different, the numerical values of plasma glucose TR of the present findings were comparable to the data reported in sheep fed plantain herb (Al-Mamun et al., 2007).

Research on protein metabolism in sheep fed the RSUM-diet is scanty. In the present study, although N intake was lower for the RSUM-diet than the MH-diet, plasma LeuTR as well as WBPS and WBPD did not differ between diets. This might be due to presence of urea and molasses in the RSUM-diet which accelerated the microbial protein synthesis in the rumen. This is in agreement with Rooke and Armstrong (1989),

who pointed that availability of soluble carbohydrates in the diets influenced the microbial protein synthesis in the rumen. Moreover, Sano et al. (2009) reported that supplementation of urea and SBM to roughage-based diets did not influence the plasma LeuTR as well as WBPS and WBPD in sheep. The numerical values of plasma LeuTR as well as WBPS and WBPD were slightly improved than the data previously found in sheep fed rice straw only (**Chapter1**), because the N intake and estimated ME intake were also greater in sheep fed the RSUM-diet than rice straw only diet.

From the current results it can be said that the untreated rice straw supplemented with urea and molasses was comparable to mixed hay for the metabolism of plasma glucose and protein in sheep. It is hoped that ensiling of rice straw with urea and molasses will improve the kinetics of plasma nutrient metabolism in sheep through providing NH_3 and readily fermentable energy substrates due to proper fermentation in ensiling period. Further research should be performed to investigate the effects of rice straw-based silage on intermediary metabolism of plasma nutrients in sheep.

Chapter4

Intermediary Metabolism of Plasma Acetate, Glucose and Leucine Kinetics in Sheep Fed Rice Straw Supplemented with Urea and Molasses Silage

Introduction

The previous studies (**Chapter2** and **Chapter3**) proved that untreated rice straw supplemented with nitrogenous substrates and energy sources improved digestive function and ruminal characteristics, but did not influence the intermediary metabolism of plasma nutrient in sheep. This is because the high fiber content in rice straw that makes it difficult for sheep to digest in untreated form (Orden et al., 2000). Ensiling is regarded as a good forage preservation method, because fermentation by some microbes is an effective way to improve the digestibility, palatability and nutritive values of straws (Holmes et al., 1987; Nishino et al., 2004; Gao et al., 2008). Various additives such as urea, molasses, ammonia, inoculants and acids have been tested to increase the quality of silage or to improve the ruminal fermentation (Takahashi et al., 2005; Mahala and Khalifa, 2007; Zhang et al., 2010).

It can be expected that rice straw supplemented with urea and molasses silage (RSUMS-diet) will improve the metabolism of plasma acetate, glucose and Leu in sheep through influencing ruminal microbial activities. However, until now, the effects of a RSUMS-diet on intermediary metabolism of plasma acetate, glucose and Leu has not been investigated. Therefore, the current study was undertaken to find out the

feeding effects of a RSUMS-diet on plasma acetate, glucose and Leu metabolism in sheep using combined experiments of isotope dilution methods and N balance test.

Materials and Methods

Animals, diets and management

Four crossbred (Correidale x Suffolk) adult shorn sheep averaging 50.2±2.1 kg of BW at the begining of the study were used in this experiment. The sheep were assigned to two dietary treatments including either a RSUMS-diet or a MH-diet using 100% ME for maintenance. Chemical composition of experimental feeds is presented in **Table 4.1.** The ME was estimated at 0.91 kcal/g for the RSUMS-diet, and 1.73 kcal/g for the MH-diet, according to the same feeding standard as mentioned before (**Chapter1**). The feed allowance was rice straw-based silage 109.9 g/kg$^{0.75}$/d for the RSUMS-diet and mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet, as fed basis. Crude protein supply was 5.4 g/kg$^{0.75}$/d for the RSUMS-diet and 6.4 g/kg$^{0.75}$/d for the MH-diet, on DM basis. The animals were fed twice daily at 11:00 h and 19:00 h, and fresh drinking water was available *ad libitum*. The experiment was performed using a crossover design with two 21 d periods. Two sheep were fed the RSUMS-diet during the first period, and then the MH-diet during the second period, and the other two sheep were fed in the reverse order. The sheep were housed in individual pens in a sheep barn during the adjustment period (the first two weeks of the experiment). On d 15, sheep were moved to controlled environment house with air temperature 23±1 °C, 70% relative humidity and lighting from 7:00 h to 21:00 h and maintained in wooden metabolic cages for total collection of urine and feces. The BW of the sheep was measured on d 1, d 8, d 15 and d 21 of each

dietary treatment. The handling of animals including cannulation and blood sampling was carried out as described in **Chapter1.** The schematic diagram of experimental layout is shown in **Figure 4.1**.

Table 4.1. Chemical composition of experimental feed on the basis of air dry matter

Items (%)	MH	RSUMS
Dry matter	87.9	48.0
Crude protein	12.7	10.2
Crude ash	10.8	11.2
NDF	69.4	52.4
ADL	2.0	2.2

MH = Mixed hay of orchardgrass and reed canarygrass, RSUMS = Rice straw supplemented with urea and molasses silage, NDF = Neutral detergent fiber, ADL = Acid detergent lignin.

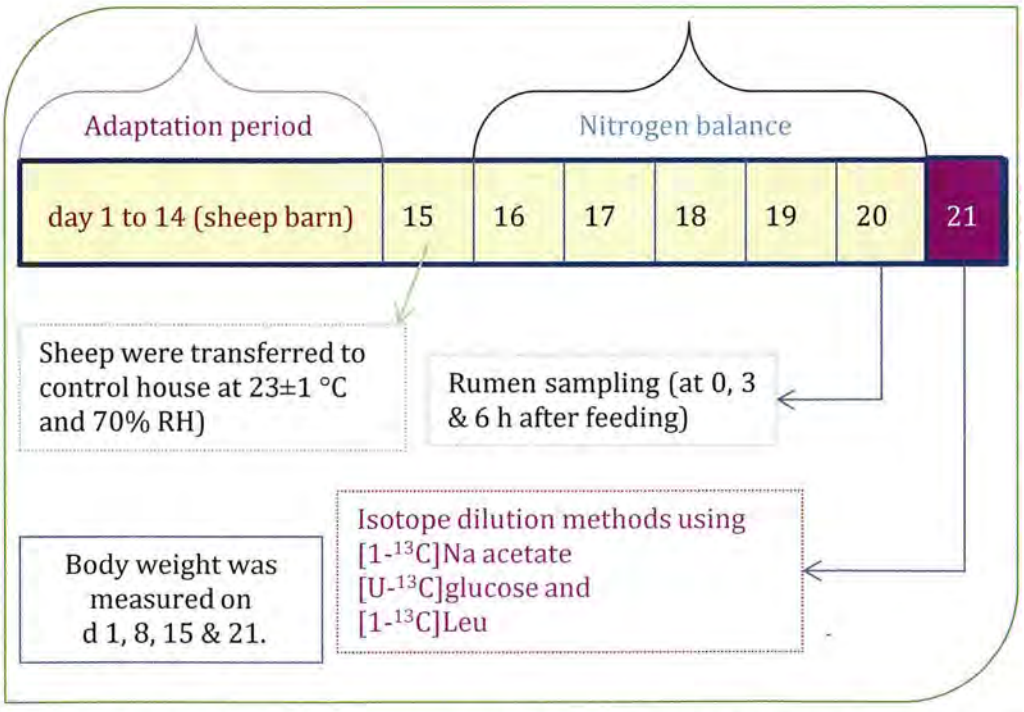

Figure 4.1. Schematic diagram of experimental layout showing the sampling protocols

Preparation of silage

Prior to processing, the rice straw was chopped in a particular (3-4 cm) size and spreaded on a clean polythene sheet. Urea (1.5 % of DM of rice straw) and molasses (10 % of DM of rice straw) were dissolved with required amount of water to keep the moisture content of silage at about 50-60%. The well mixed urea and molasses solution was then sprayed over the chopped rice straw by hand and mixed properly. The prepared materials were then put into the plastic silo (180 L), pressed sufficiently to create anaerobic conditions, filled properly and kept for 21 d for better fermentation. After 21 d processing the silo was opened and silage was offered to the animals. The silage containing silo and silage for chemical analysis are shown in **Figure 4.2**.

Figure 4.2. An airtied silo and some portion of silage after 21 d fermentation for chemical analysis

Preparation of silage extracts

After finishing the 21 d ensiling period, the silo was opened for offering to the animals and a portion of the silage was taken from different places for making silage

extracts. For preparing the silage extracts, 15 g DM of silage was put into a beaker, added 140 ml of distilled water, and put into the refrigerator for 24 h for dissolving. The extract was collected using filter cloth (gauze) with hand pressure from the well dissolved sample. Immediately after collection the extract, pH was measured by the pH meter and then preserved at -30 °C for further analysis of volatile basic N (VBN), lactic acid and VFA.

Nitrogen balance

A five d (from d 16 to d 20) long N balance trial was conducted for each dietary treatment in which urine, feces and feed refusals were collected. The samples of urine, feces and feed refusals were treated using the procedure as described before in **Chapter1**.

Rumen fluid collection

On d 20 of each dietary period rumen fluid was collected at 0, 3 and 6 h after the morning feeding via an orally inserted stomach tube for measuring the pH, NH_3 and VFA. The pH of the rumen fluid was measured immediately after collection by the pH meter. A sub-sample was centrifuged and then an aliquot of supernatant was acidified by 0.1 N HCl for measuring rumen NH_3 concentration as described in **Chapter1**. The acidified supernatant and residuals were stored at -30 °C for further analysis.

Isotope dilution method

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 to determine the TR of plasma acetate, glucose, and Leu in sheep. Isotope dilution method, rate of isotope

infusion and blood sampling procedures were the same as described in **Chapter1.** After the end of the experiment plasma was separated from blood cells by centrifuging as before (**Chapter1**) and supernatant was then stored at -30 °C for later analysis.

Chemical analysis

Analysis of N in diets, feces, urine and feed refusals and chemical composition of experimental feed is same as before (**Chapter1**). The fermentation products of silage were analyzed using cold-water extract (Cai et al., 1999). Lactic acid concentration of silage was measured by the colorimetric method (Taylor, 1996). The VBN content of the silage was determined according to steam distillation method as described previously (Dhaouadi et al., 2007). Concentrations of NH_3 and VFA in rumen fluid, and concentrations of VFA in silage were determined using the procedure as described in **Chapter1**. To evaluate the quality of silage, the Fleig point was calculated from the relationship between DM and pH values of silage according to Yilmaz et al. (2009), and the V-score was calculated from the relationship of VBN/total N and VFA concentrations as described previously by Takahashi et al. (2005).

In pre-infusion of isotope, plasma free AA, NH_3, urea and NEFA were determined using the procedure described in **Chapter1**. Plasma [1-^{13}C]acetate enrichment and concentrations of plasma VFA and lactate were determined using the selected ion monitoring with the GC/MS. Concentrations of plasma glucose and [U-^{13}C]glucose enrichment were determined by the procedure described previously (**Chapter1**). Plasma AA and α-keto acids were separated and converted to MTBSTFA derivatives according to the procedures described in **Chapter1**. Isotopic enrichments of plasma [1-^{13}C]Leu

and α-[1-^{13}C]KIC and concentrations of plasma Leu and α-KIC were measured by the selected ion monitoring system using the GC/MS.

Calculation

Results are presented as mean values with SEM. 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, 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 isotopic enrichment of [1-^{13}C]acetate, [U-^{13}C]glucose and [1-^{13}C]Leu or α-[1-^{13}C]KIC at steady state.

The WBPS and WBPD were calculated from the relationship among WBPF, LeuTR and N balance according to the equations described in **Chapter1** as follows:

$$WBPF = LeuTR/0.066$$

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

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

Statistical analysis

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

Results

Silage quality and N balance

Data of rice straw-based silage quality are presented in **Table 4.2**. Dry matter intake, estimated ME intake and BW gain did not differ between diets (**Table 4.3**). Animals were in positive N balance for both diets. Nitrogen intake and N excretion through feces were lower ($P < 0.0001$) for the RSUMS-diet than the MH-diet. Nitrogen excretion through urine was considerably higher ($P = 0.01$) for the RSUMS-diet compared to the MH-diet which resulted in lower ($P = 0.01$) N retention for the RSUMS-diet than the MH-diet. The lower N excretion through feces increased ($P = 0.001$) the N digestibility for the RSUMS-diet than the MH-diet.

Table 4.2. Fermentative characteristics of rice straw treated with urea and molasses

Items	RSUMS
pH	4.5
Moisture (g/kg FM)	520
Lactic acid (g/kg FM)	42
Acetate (g/kg FM)	3.1
Propionate (g/kg FM)	0.1
Butyrate (g/kg FM)	0.02
VBN (g/kg FM)	0.2
V-score	89
Flieg point	100

RSUMS = Rice straw supplemented with urea and molasses silage,
VBN = Volatile basic nitrogen, FM = Fresh matter.

Rumen fermentation caharacteristics

Rumen pH did not differ between diets (**Table 4.4.**). Rumen NH_3 concentration was higher ($P = 0.004$) for the RSUMS-diet than the MH-diet. Concentrations of rumen total

VFA and acetate did not differ between diets. Concentration of propionate in the rumen tended to be higher (P = 0.07), iso-butyrate and iso-valerate were lower (P < 0.05) for the RSUMS-diet compared to the MH-diet. Concentrations of butyrate and valerate in the rumen did not differ between dietary treatments.

Table 4.3. Dietary effects on body weight (BW) gain, dry matter (DM) intake, estimated metabolizable energy (ME) intake, nitrogen (N) balance and N digestibility in sheep

Items	MH-diet	RSUMS-diet	SEM	*P*-value
No. of sheep	4	4		
BW gain (g/d)	92	31	63	0.15
DM intake (g/kg$^{0.75}$/d)	50	51	1	0.76
ME intake (kcal/kg$^{0.75}$/d)	99	97	1	0.41
N intake (g/kg$^{0.75}$/d)	1.04	0.88	0.05	<0.0001
N in feces (g/kg$^{0.75}$/d)	0.38	0.24	0.04	<0.0001
N in urine (g/kg$^{0.75}$/d)	0.48	0.61	0.04	0.01
N absorption (g/kg$^{0.75}$/d)	0.66	0.64	0.03	0.0001
N retention (g/kg$^{0.75}$/d)	0.18	0.03	0.05	0.01
N digestibility (%)	64	72	3	0.001

MH = Mixed hay of orchardgrass and reed canarygrass, RSUMS = Rice straw supplemented with urea and molasses silage, SEM = Standard error of the mean.

Plasma metabolites

Plasma total free AA concentrations were lower (P = 0.01) for the RSUMS-diet than the MH-diet (**Table 4.5**). Among the individual plasma free AA, the concentrations of plasma methionine, lysine, aspartic acid, glutamic acid and glutamine were higher (P < 0.05), and concentrations of valine, Leu, phenylalanine, tyrosine, tryptophan and arginine were lower (P < 0.05) for the RSUMS-diet compared to the MH-diet. Concentration of plasma NH_3 tended to be higher (P = 0.05) and urea was higher

(P = 0.03) for the RSUMS-diet than the MH-diet. Plasma lactate and NEFA concentrations did not differ between diets.

Table 4.4. Dietary effects on rumen pH, concentrations of rumen ammonia (NH_3) and volatile fatty acid (VFA) at 0, 3 and 6 h after feeding in sheep

Items	Treatments						SEM	*P*-value		
	MH-diet			RSUMS-diet						Diet
	0	3	6	0	3	6		Diet	Time	×
No. of sheep	4	4	4	4	4	4				Time
pH	6.94	6.74	6.67	6.87	6.78	6.82	0.09	0.70	0.10	0.50
(mmol/L)										
NH_3	6.1^{e}	6.3^{d}	5.0^{f}	7.6^{c}	13.5^{a}	12.8^{b}	1.8	0.004	0.01	0.01
Total VFA	85.6	95.1	94.5	87.2	88.6	98.1	5.5	0.92	0.24	0.60
Acetate	66.1	73.3	73.7	66.4	65.0	71.7	4.1	0.38	0.33	0.54
Propionate	13.8	15.2	15.1	16.0	18.3	20.4	1.4	0.07	0.15	0.56
Iso-Butyrate	0.6	0.6	0.5	0.4	0.3	0.3	0.04	0.01	0.26	0.18
Butyrate	4.1	4.9	4.5	3.7	4.4	5.1	0.4	0.64	0.04	0.22
Iso-Valerate	0.8	0.7	0.4	0.4	0.3	0.3	0.1	0.04	0.10	0.10
Valerate	0.3	0.4	0.3	0.3	0.4	0.3	0.04	0.40	0.40	0.77

MH = Mixed hay of orchardgrass and reed canarygrass, RSUMS = Rice straw supplemented with urea and molasses silage, SEM = Standard error of the mean,
[a, b, c] Values on the RSUMS-diet with different superscripts differ (P < 0.05),
[d, e, f] Values on the MH-diet with different superscripts differ (P < 0.05)

Plasma acetate, glucose and Leu kinetics

Plasma acetate concentration and enrichment of plasma [1-^{13}C]acetate remained constant during the latter half of the [1-^{13}C]Na acetate infusion (**Figure 4.3**). Plasma acetate concentration calculated during the last 2 h continuous infusion of the isotope dilution did not differ between diets (**Table 4.6**). Turnover rate of plasma acetate determined from the enrichment of [1-^{13}C]acetate did not differ between dietary treatments.

Table 4.5. Dietary effects on plasma metabolite concentrations in sheep

Items	MH-diet	RSUMS-diet	SEM	*P*-value
No. of sheep	4	4		
Essential AA (μmol/L)				
Threonine	219	185	41	0.15
Valine	251	150	41	0.01
Methionine	16	23	3	0.04
Iso-leucine	94	63	16	0.06
Leucine	132	74	23	0.01
Phenylalanine	48	39	4	0.03
Histidine	26	21	2	0.10
Lysine	33	40	3	0.03
Nonessential AA (μmol/L)				
Aspartic acid	9	11	2	0.02
Serine	180	178	15	0.18
Asparagine	80	67	9	0.05
Glutamic acid	290	379	35	0.03
Glutamine	80	172	27	0.04
Glycine	623	571	44	0.13
Alanine	211	171	25	0.08
Tyrosine	66	60	8	0.01
Tryptophan	114	103	20	0.01
Arginine	144	110	15	0.003
Proline	62	53	18	0.60
Total AA (μmol/L)	2675	2301	178	0.01
NH3 (μmol/L)	385	461	46	0.05
Urea (mmol/L)	6.9	11.1	1.8	0.03
Lactate (μmol/L)	233	222	22	0.54
NEFA (μEq/L)	109	168	30	0.11

MH = Mixed hay of orchardgrass and reed canarygrass, RSUMS = Rice straw supplemented with urea and molasses silage, SEM = Standard error of the mean, AA = Amino acid, NEFA = Non-esterified fatty acid.

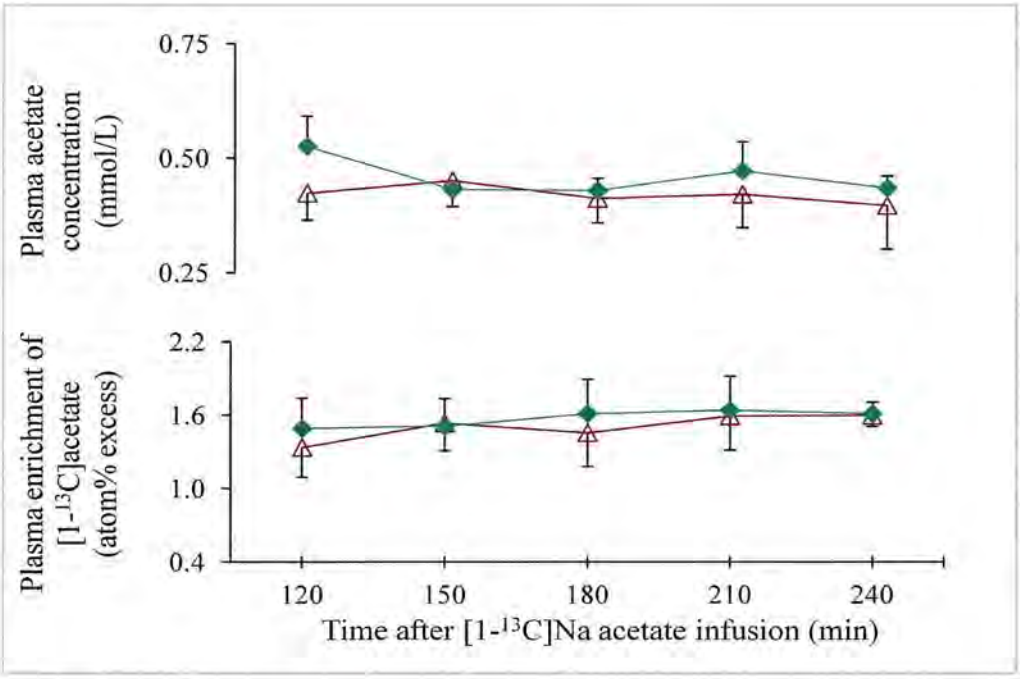

Figure 4.3. Time course changes in plasma acetate concentrations and plasma enrichments of [1-^{13}C]acetate during the last 2 h continuous infusion of [1-^{13}C]Na acetate in sheep fed the RSUMS-diet (Δ) and the MH-diet (♦).

Plasma glucose concentration and enrichment of plasma [U-^{13}C]glucose remained constant during the latter half of the [U-^{13}C]glucose infusion (**Figure 4.4**). Concentration of plasma glucose determined during the latter half of the primed-continuous infusion of isotope dilution remained unchanged between diets (**Table 4.6**). Turnover rate of plasma glucose calculated from the enrichment of [U-^{13}C]glucose did not differ between the RSUMS-diet and the MH-diet.

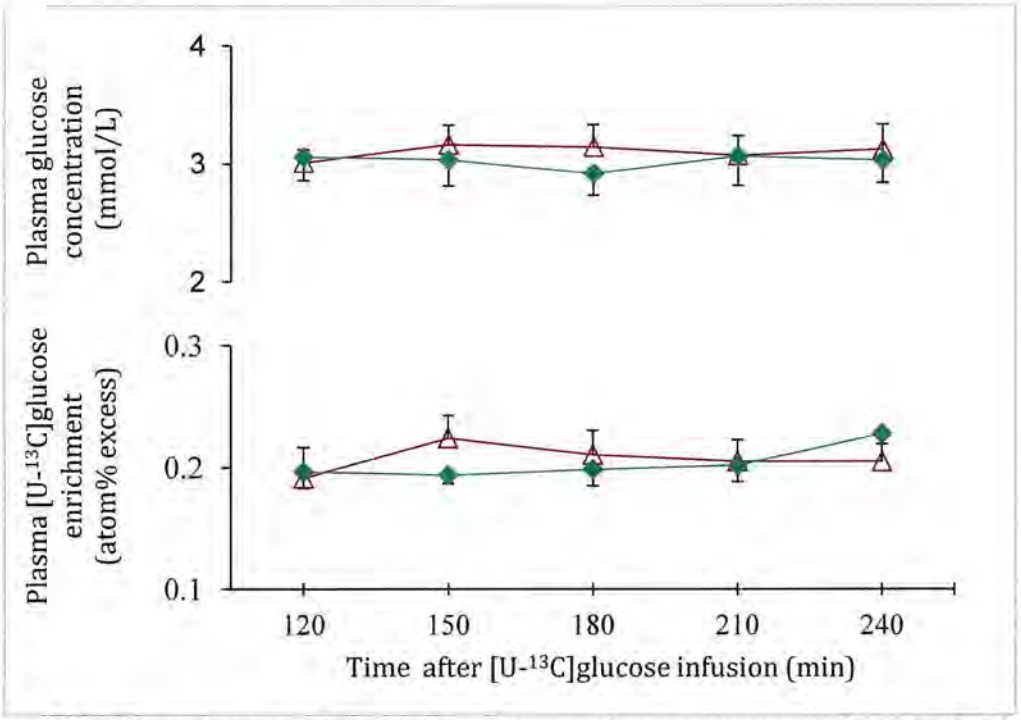

Figure 4.4. Time course changes in plasma glucose concentrations and plasma enrichments of [U-^{13}C]glucose during the last 2 h continuous infusion of [U-^{13}C]glucose in sheep fed the RSUMS-diet (Δ) and the MH-diet (♦).

Concentration of plasma Leu and enrichment of plasma [1-^{13}C]Leu remained constant during the latter half of [1-^{13}C]Leu infusion (**Figure 4.5**). Plasma α-KIC concentration and enrichment of α-[1-^{13}C]KIC remained constant during the latter half of [1-^{13}C]Leu infusion (**Figure 4.6**). Plasma Leu concentration calculated during the last 2 h of isotope infusion did not differ between dietary treatments, and plasma α-KIC concentration tended to be lower (P = 0.06) for the RSUMS-diet than the MH-diet (**Table 4.6**). Plasma LeuTR as well as WBPS and WBPD determined from the enrichment of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC did not differ between dietary treatments.

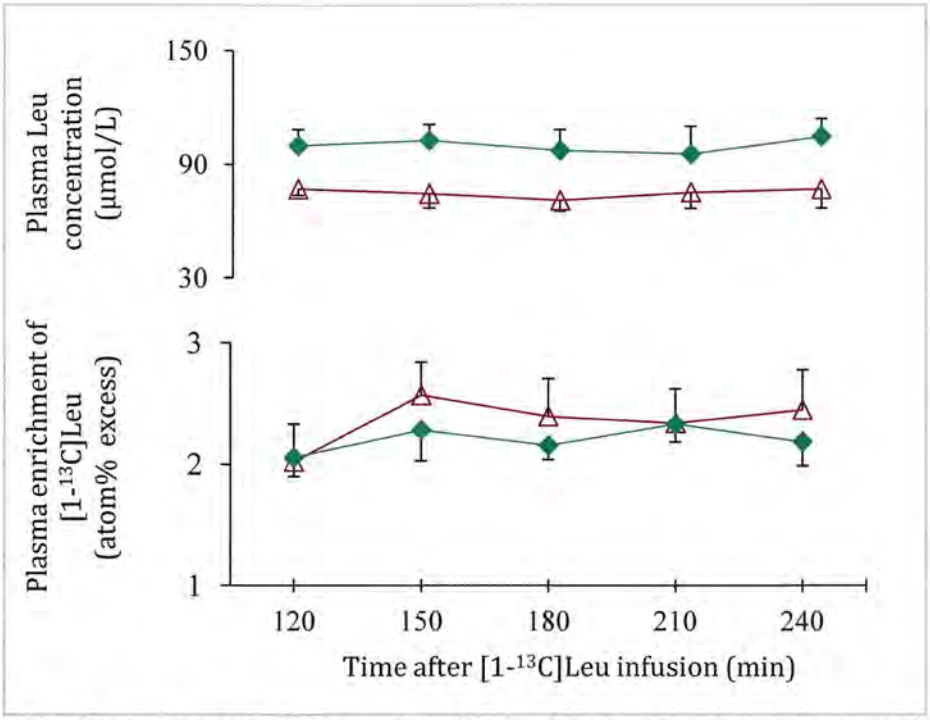

Figure 4.5. Time course changes in plasma leucine concentrations and plasma enrichments of [1-^{13}C]Leu during the last 2 h continuous infusion of [1-^{13}C]Leu in sheep fed the RSUMS-diet (Δ) and the MH-diet (♦).

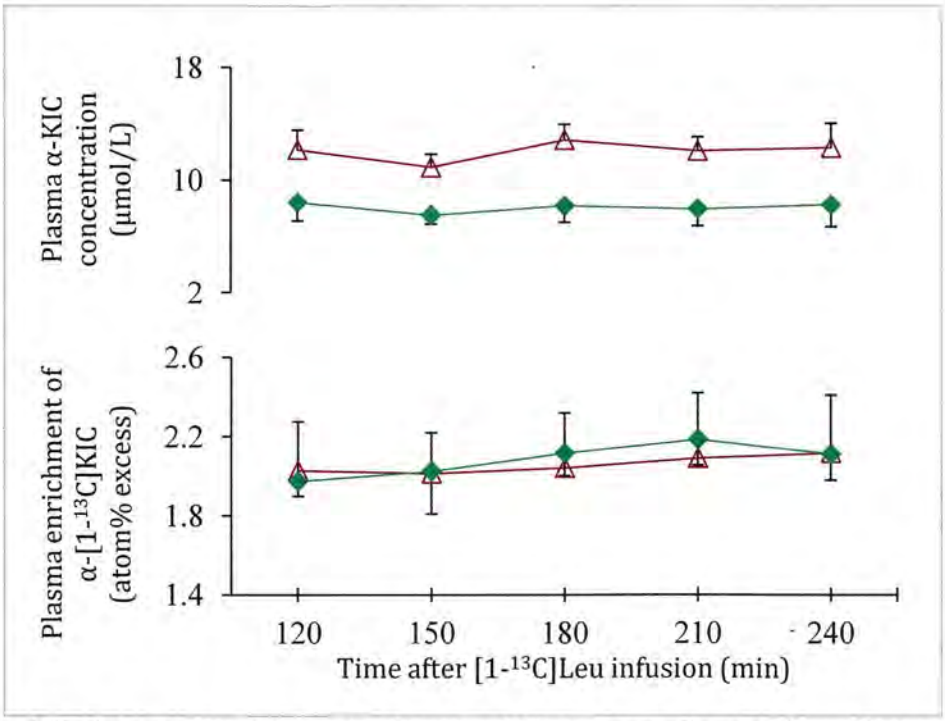

Figure 4.6. Time course changes in plasma α-KIC concentrations and enrichments of plasma α-[1-^{13}C]KIC during the last 2 h continuous infusion of [1-^{13}C]Leu in sheep fed the RSUMS-diet (Δ) and the MH-diet (♦).

Table 4.6. Dietary effects on kinetics plasma acetate, glucose and leucine (Leu) metabolism in sheep

Items	MH-diet	RSUMS-diet	SEM	*P*-value
No. of sheep	4	4		
Acetate				
Concentration (μmol/L)	474	431	28	0.35
TR (mmol/kg$^{0.75}$/h)	5.55	5.71	0.68	0.84
Glucose				
Concentration (mmol/L)	3.04	3.11	0.14	0.50
TR (mmol/kg$^{0.75}$/h)	1.59	1.55	0.11	0.73
Leu				
Concentration (μmol/L)	98.4	75.4	8.7	0.15
TR (μmol/kg$^{0.75}$/h)	321	280	27	0.29
WBPS (g/kg$^{0.75}$/d)	12.3	9.4	1.4	0.19
WBPD (g/kg$^{0.75}$/d)	11.2	9.2	1.3	0.34
α-KIC				
Concentration (μmol/L)	12.1	8.1	1.6	0.06
TR (μmol/kg$^{0.75}$/h)	334	313	28	0.43
WBPS (g/kg$^{0.75}$/d)	12.9	11.1	1.4	0.20
WBPD (g/kg$^{0.75}$/d)	11.8	10.9	1.3	0.56

MH = Mixed hay of orchardgrass and reed canarygrass, RSUMS = Rice straw supplemented with urea and molasses silage, SEM = Standard error of the mean, TR = Turnover rate, WBPS = Whole body protein synthesis, WBPD = Whole body protein degradation, α-KIC = α-ketoisocaproic acid.

Discussion

Silage quality

The pH is often considered one of the crucial factors in evaluating the fermentation quality of silage (Muck et al., 1988). During the ensiling period, acetate and lactate are fermentation end products which improve silage quality (Gao et al., 2005), while butyric acid often deteriorates silage quality (McDonald et al., 1991). The rice straw-based silage fed in the present study had high lactic acid, high V-score and Fleig point

indicating that RSUMS was good quality silage and resulted from excellent fermentation in the silo. The pH value, moisture content and organic acid content were close to the values reported in whole crop rice silage (Takahashi et al., 2005).

Nitrogen balance

Lower N excretion through feces for the RSUMS-diet is in accordance with Atkinson et al. (2007), who revealed that in lambs fecal N excretion decreased linearly with increasing supplementation of nitrogenous substrates. The higher urinary N excretion for the RSUMS-diet indicates the excess NH_3 production in the rumen, because NH_3 in excess of microbial protein synthesis is converted to urea in the liver and most of the urea is excreted in the urine, and some recycled via saliva (Nolan and Leng, 1972). This agreed with the observation by Siddons et al. (1985). They reported that rapid degradability of dietary N from silage increased the urinary N excretion in sheep. A similar trend was found in sheep fed straw-based silage (Abazinge et al., 1994). In spite of lower N intake, the higher N digestibility for the RSUMS-diet might be due to ensiling of rice straw with urea and molasses which made the gradual release of NH_3 for microbial activities in the rumen through better fermentation during ensiling. This is in accordance with Broderick and Radloff (2004). The numerical values of N digestibility of the present study was comparable with the data reported previously in sheep fed urea ensiled rice straw (Yulistiani et al., 2003).

Rumen fermentation characteristics

No differences in rumen pH occured in the present study, consistent with Cameron et al. (1991), who found that rumen pH was not changed in cows fed alkaline hydrogen

peroxide treated wheat straw. The numerical values of rumen pH of the present study ranged from 6.7 to 6.9, which were in optimal pH range of 6.7±0.5 to maintain normal cellulolysis (Van Soest, 1994) and above 6.0 for microbial protein synthesis (Russel et al., 1992). The results of rumen fluid pH of the present findings were similar with the data reported in cattle and buffaloes fed rice straw supplemented with urea-molasses blocks (Wanapat et al., 1999). In the current study the mean values of NH_3 concentration in the rumen for both diets is above the minimum concentration of approximately 2.9 mmol/L for promoting desireable rumen fermentation rate (Balcells et al., 1993). Higher NH_3 concentration for the RSUMS-diet in the present study was likely due to soluble N degraded rapidly from the RSUMS-diet in the rumen. This is in agreement with previous findings (Bonsi et al., 1995; Orden et al., 2000). Dijkstra (1994) suggested that production of VFA the end product of microbial fermentation largely depended on the type of dietary carbohydrate ingested. In the present study similar rumen total VFA and acetate concentrations between diets indicated the similar fermentability for both diets in the rumen. A tendency of higher propionate concentration in the rumen for the RSUMS-diet might be due to available of molasses as source of soluble carbohydrate in the RSUMS-diet in accordance with the previous findings (Greathead et al., 2006).

Plasma metabolites

Level of dietary N intake influenced the concentrations of AA in the blood plasma, and N from different sources resulted in different plasma amino acid levels in steers and sheep (Young et al., 1973; Remond et al., 2000). In the present study plasma total free

AA concentrations were lower for the RSUM-diet. This might be due to rapid digradability of N from the RSUMS-diet. This is in accordance with Milano and Lobley (2001), who suggested that in sheep rapidly degradable N reduced the AA availability for protein metabolism. Plasma NH_3 and urea concentrations are closely related to production of NH_3 in the rumen and absorbed into portal blood as described before (**Chapter1**). Higher plasma urea concentration for the RSUMS-diet indicated the rapid absorption of NH_3 from the rumen into blood circulation and converted to urea in the liver. The present results were supported by Nolan and Leng (1972) who reported the influence of ruminal NH_3 on plasma urea in sheep. Plasma NEFA is the important nutritional status in ruminants (Tokuda et al., 2002). In the present experimental condition the similar NEFA concentration indicated the similar nutritional status between diets. Lower plasma NEFA concentration in the present study indicating that the sheep were felt comfort to consume the rice straw-based silage diet.

Kinetics of plasma acetate, glucose and Leu metabolism

The principal energy substrate in ruminants is acetate. The concentration of acetate in blood circulation is inversely related to the concentration of acetate in the rumen (Pethick et al., 1981; Cronje et al., 1991). This is in accordance with the present study. Plasma acetate concentration was similar between diets, this is because the similar acetate concentration in the rumen for both diets. Plasma acetate TR depends largely on acetate production in the rumen and absorbed into portal vein as described previously (**Chapter1**). Plasma acetate TR did not differ between diets in the present study. This implies that animals fed the RSUMS-diet and the MH-diet had similar acetate

absorption rates from rumen wall to portal blood, and under steady-state condition it is assumed to be equal acetate TR for both diets. This is in accordance with Greathead et al. (2006) who did not find significant difference for plasma acetate TR in steers fed grass silage and dried grass. The numerical values of plasma acetate TR of the present study were comparable to the data reported in sheep fed ensiled hop residues (Al-Mamun et al., 2011). Moreover, numerical values of plasma acetate TR of the present study were slightly improved than the data previously shown in sheep fed rice straw only diet (**Chapter1**), and in sheep fed rice straw supplemented with SBM without ensiling (**Chapter2**). It is indicated that ensiling rice straw with urea and molasses, ensured the better fermentation of dietary carbohydrate in the rumen through influencing the microbial activities.

Kinetics of glucose metabolism has not been investigated yet in sheep fed ensiling rice straw supplemented with urea and molasses. Plasma glucose concentrations of the present findings were comparable to data reported in lactating dairy cows fed corn silage diet (Polat et al., 2009). Whole body glucose TR depends on the dietary carbohydrate type and mainly starch rich diet is responded positively on glucose metabolism. Dietary energy intake and precursor availability were important factors for regulating the gluconeogenesis as described previously in **Chapter3**. Similar plasma glucose TR between the diets in the present study might be due to the similar fermentability pattern and dietary energy intake level between diets. The present results were supported by the previous findings of Konig et al. (1984), who revealed that the dietary energy intake level was responsible to regulate the glucose metabolism in

lactating cows. The numerical values of plasma glucose TR in the present study were close to the data reported in sheep fed plantain herb diet (Al-Mamun et al., 2007), although they used the single injection isotope dilution method. Moreover, the values of plasma glucose TR of the present study slightly higher than the data found in sheep fed rice straw only (**Chapter1**). This may be due to the well fermentation of dietary carbohydrate of the RSUMS-diet through ensiling.

Kinetics of plasma Leu metabolism has not been investigated in sheep fed rice straw-based silage. In the previous experiment (**Chapter3**) plsama Leu metabolism was not influenced in sheep consuming untreated rice straw supplemented with urea and molasses. Lobley et al. (1987) revealed that the plsama LeuTR was positively associated with protein addition rates in beef steers. Savary-Auzeloux et al. (2003) demonstrated that in sheep the whole body protein metabolism was associated with dietary ME intake. In my present study plasma LeuTR determined from the enrichments of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC remained similar between diets, although N intake was lower for the RSUMS-diet than the MH-diet. This is probably due to similar estimated ME intake for both diets in the present study, because plasma LeuTR in sheep was influenced slightly by dietary CP intake when ME intake was constant as decribed in **Chapter1**. Thus it is noted that the RSUMS-diet did not adversely affect the plasma LeuTR in sheep in the present study.

While there was difference in CP content between the diets, the lower CP on DM basis of the RSUMS-diet resulted in lower N intake for the RSUMS-diet. Lower N intake for the RSUMS-diet did not depress the WBPS and WBPD in the present study.

This is in accordance with the previous results found by Sano et al. (2004). The present results were also supported by Greathead et al. (2006), who determined the whole body protein metabolism in steers fed grass silage and dried grass did not find significant difference between diets. The numerical values of plasma LeuTR as well as WBPS and WBPD were slightly higher than the data observed in sheep fed rice straw only diet (**Chapter1**). The comparable performance was found between the RSUMS-diet and the MH-diet on kinetics of plasma acetate, glucose and Leu in the present study. Thus ensiling rice rice straw supplemented with nitrogenous substrates and soluble carbohydrate source can serve as an alternative of mixed hay for ruminant production.

Chapter5

Summary and Conclusion

Short background

World population is increasing day by day and increasing the demand of ruminants production to meet meat and milk requirements to improve the quality of life. However, feed shortage is a major constraint to raise livestock production, especially in tropical and sub-tropical countries it is a severe problem. Rice straw can play an important role for raising livestock production due to its abundant availability throughout the world. The present study has been conducted to evaluate the feeding value of rice straw-based different diets through treating or supplementing with nitrogenous and energy substrates on intermediary metabolism of plasma nutrient in sheep using stable isotope dilution techniques as follows:

Experiment-1

First of all, the combined experiment of isotope dilution methods using [1-^{13}C]Na acetate, [U-^{13}C]glucose, [1-^{13}C]leucine (Leu), and nitrogen (N) balance test were conducted to determine the effects of rice straw only (RS-diet) on intermediary metabolism of plasma acetate, glucose and Leu in sheep. Four sheep were assigned to two dietary treatments including either mixed hay (MH-diet) or a RS-diet using crossover design with two 21 d periods. The sheep were fed either mixed hay 40.5 g/kg$^{0.75}$/d for the MH-diet, or rice straw 67.2 g/kg$^{0.75}$/d for the RS-diet. The isotope dilution methods were conducted as the primed-continuous infusion on d 21 of each dietary period. Nitrogen intake and N digestibility were considerably lower ($P < 0.05$)

for the RS-diet compared to the MH-diet. Rumen ammonia (NH_3) concentration was lower ($P < 0.05$) for the RS-diet than the MH-diet. Turnover rates (TR) of plasma acetate and glucose did not differ between diets, but numerical values were slightly lower for the RS-diet than the MH-diet. Plasma LeuTR as well as whole body protein synthesis (WBPS) and degradation (WBPD) did not differ between diets, but numerical values were lower for the RS-diet compared to the MH-diet.

Experiment-2

From the **Experiment-1** it was found that although not significant, turnover rates of plasma acetate, glucose and Leu as well as WBPS and WBPD were slightly lower in sheep fed rice straw compared to mixed hay. It could be expected that supplementation of protein or energy source to rice straw would improve the plasma nutrient metabolism through providing required NH_3 and energy for rumen microbes. Thus **Experiment-2** was designed using an isotope dilution method of [1-^{13}C]Na acetate to assess the feeding effects of rice straw supplemented with soybean meal (SBM) as a high energy protein source (RSS-diet) on intermediary metabolism of plasma acetate kinetics in sheep compared with a MH-diet. Four sheep were assigned either a MH-diet or a RSS-diet using crossover design with two 24 d periods. The isotope dilution method was conducted as the primed-continuous infusion on d 24 of each dietary period. The sheep were fed either mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet or rice straw 59.4 g/kg$^{0.75}$/d supplemented with SBM 7.4 g/kg$^{0.75}$/d for the RSS-diet. Nitrogen intake did not differ between diets, and N digestibility was considerably higher ($P < 0.05$) for the RSS-diet compared to the MH-diet. Concentration of acetate in the rumen did not differ between

diets. Plasma acetate concentration as well as turnover rate of plasma acetate did not differ between the RSS-diet and the MH-diet. From the current study, it was proved that supplementation of SBM as high energy protein source showed comparable performance to mixed hay on intermediary metabolism of plasma acetate in sheep.

Experiment-3

The **Experiment-2** showed that rice straw supplemented with SBM gave the comparable performance to mixed hay on plasma acetate metabolism in sheep. It could be expected that supplementation of protein source along with soluble carbohydrate source would improve the intermediary metabolism of plasma nutrients through providing required nitrogenous substrates and easily fermentable energy substrates for microbial growth. Therefore, **Experiment-3** was designed using isotope dilution methods of [U-^{13}C]glucose and [1-^{13}C]Leu to investigate the effects of rice straw supplemented with urea and molasses (RSUM-diet) on intermediary metabolism of plasma glucose and Leu kinetics in sheep. Four sheep were fed either a MH-diet or a RSUM-diet using crossover design with two 21 d periods. The isotope dilution methods were conducted as the primed-continuous infusion on d 21 of each dietary period. Feed allowance was computed on the basis of metabolizable energy (ME) at maintenance level. The sheep were fed either mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet or rice straw 59.7 g/kg$^{0.75}$/d supplemented with urea 0.84 g/kg$^{0.75}$/d and molasses 7.6 g/kg$^{0.75}$/d for the RSUM-diet. Nitrogen intake was lower ($P < 0.05$) for the RSUM-diet than the MH-diet, but N digestibility remained similar between diets. Concentrations of NH_3 and propionate in the rumen were higher ($P < 0.05$) for the RSUM-diet than the MH-diet.

Turnover rate of plasma glucose did not differ between diets, but numerical value was slightly higher for the RSUM-diet than the MH-diet. Plasma LeuTR as well as WBPS and WBPD were comparable between the RSUM-diet and the MH-diet.

Expeiment-4

From the **Experiment-2** and **Experiment-3** it was found that untreated rice straw supplemented with nitrogenous substrates and energy substrates showed the comparable performance to mixed hay on intermediary metabolism of plasma nutrients in sheep. It is expected that ensiling rice straw supplemented with nitrogenous substrates and energy source will influence the intermediary metabolism of plasma nutrients through better fermentation. Thus **Experiment-4** was designed using the isotope dilution methods of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]Leu to evaluate the feeding effects of rice straw supplemented with urea and molasses silage (RSUMS-diet) on intermediary metabolism of plasma acetate, glucose and Leu in sheep. Four sheep were assigned to either a MH-diet or a RSUMS-diet using a crossover design with two 21 d periods. The feed allowance was mixed hay 57.8 g/kg$^{0.75}$/d for the MH-diet, and rice straw-based silage 109.9 g/kg$^{0.75}$/d for the RSUMS-diet, as fed basis. Crude protein supply was 6.4 g/kg$^{0.75}$/d for the MH-diet, and 5.4 g/kg$^{0.75}$/d for the RSUMS-diet on DM basis. Nitrogen intake was lower ($P < 0.05$) for the RSUMS-diet compared to the MH-diet, but N digestibility was considerably higher ($P < 0.05$) for the RSUMS-diet than the MH-diet. Rumen total VFA and acetate concentrations did not differ between diets, and propionate concentration in the rumen tended to be higher ($P = 0.07$) for the RSUMS-diet than the MH-diet. Turnover rates of plasma acetate and glucose did not differ

between diets. Plasma LeuTR as well as WBPS and WBPD were similar between diets. From the results of this study, it was shown that ensiling rice straw supplemented with urea and molasses can be used for raising ruminant production as like as mixed hay.

Conclusion

Taken together the results of the present findings, it could be concluded that untreated or treated rice straw with nitrogenous substrates and energy substrates were comparable to mixed hay in relation to plasma acetate, glucose and protein metabolism in sheep.

Further implication

From the results obtained in the present research, it can be suggested that rice straw is one of the most important by-products as ruminant feed. The use of rice straw is a promising approach to overcome the problem of global feed scarcity, and a better way of recycling this residue to human nutrition through animal feeding. Effective use of rice straw will benefit livestock production. Thus untreated or treated rice straw with easily available protein or energy source should be used extensively for raising ruminant production, reducing feed cost and making a sustainable global environment.

Summary in Japanese

ヒツジにおける血漿栄養素代謝動態に及ぼす稲わら主体飼料の影響

背景

全世界の総人口は日々増加しており、生活の質向上のため肉や乳の要求量を満たすための反芻家畜生産の需要が増加している。しかしながら、飼料不足が家畜生産向上の主要な障害となっており、特に熱帯および亜熱帯地域では深刻な問題である。稲わらは、世界的に大量に供給できるため、家畜生産向上に重要な役割を果たすことができる。本研究は、ヒツジにおいてサイレージ処理あるいは窒素化合物やエネルギー基質添加の稲わら主体飼料の価値を安定同位体希釈法の血漿栄養素代謝動態から評価するために実施された。

実験１

最初に、ヒツジにおける血漿酢酸、グルコースおよびロイシン(Leu)代謝に及ぼす稲わら単独給与(RS 飼料)の影響を測定するため、[1-^{13}C]酢酸 Na、[U-^{13}C]グルコース、[1-^{13}C]Leu の同位元素希釈法および窒素(N)出納試験が実施された。ヒツジ４頭が混播牧草(MH 飼料)と RS 飼料に割り分けられ、実験は１期 21 日間のクロスオーバー法により実施した。飼料給与量は MH 飼料が混播牧草 40.5 g/kg$^{0.75}$/d、RS 飼料が稲わら 67.2 g/kg$^{0.75}$/d とした。それぞれの飼料給与 21 日目に同位元素希釈法の primed-continuous infusion 法を実施した。N 摂取量および N 消化率は MH 飼料に比べて RS 飼料が低かった($P < 0.05$)。ルーメンアンモニア(NH_3)濃度は MH 飼料に比べて RS 飼料が低かった($P < 0.05$)。血漿酢酸およびグルコース代謝回転速度(TR)は両飼料で差がなかったが、数値的には MH 飼料に比べて RS 飼料がやや低かった。血漿 LeuTR および全身のタンパク質合成速度(WBPS)および分解速度(WBPD)は飼料間に差がなかったが、数値的には MH 飼料に比べて RS 飼料が低かった。

実験２

実験１では、有意差は認められなかったものの、血漿 AceTR、GIuTR、LeuTR、WBPS、WBPD は MH 飼料に比べて RS 飼料がやや低かった。稲わらにタンパク質およびエネルギー源を添加すると、ルーメン微生物の活動に必要な NH_3 やエネルギーが供給されるため血漿栄養素代謝の改善が期待される。そこで、実験２では[1-^{13}C]酢酸 Na の同位元素希釈法を行い、ヒツジにおいて大豆粕(SBM)添加稲わら(RSS 飼料)と MH 飼料の血漿酢

酸代謝を比較した。ヒツジ４頭が１期 24 日のクロスオーバー法にしたがって MH 飼料あるいは RSS 飼料に振り分けられた。それぞれの飼料給与 24 日目に同位元素希釈法の primed-continuous infusion 法を実施した。飼料給与量は MH 飼料が混播牧草 57.8 $g/kg^{0.75}/d$、RSS 飼料が稲わら 59.4 $g/kg^{0.75}/d$、SBM 7.4 $g/kg^{0.75}/d$ とした。実験は１期 24 日間のクロスオーバー法にしたがって実施した。N 摂取量は飼料間で差がなく、N 消化率は MH 飼料に比べて RSS 飼料がかなり高かった($P = 0.004$)。血漿 AceTR および酢酸濃度は RSS 飼料と MH 飼料に差はなかった。本実験の結果から、ヒツジにおいて高エネルギー・高タンパク質である SBM を稲わらに添加すると血漿酢酸代謝動態は混播牧草に匹敵することが示された。

実験３

実験２では SBM を添加した稲わらを給与したヒツジの血漿酢酸代謝は混播牧草に匹敵することを示した。可溶性炭水化物を含むタンパク質源の添加は、微生物の成長にとって必要な窒素化合物と易発酵性エネルギー基質を供給することによって血漿栄養素代謝を改善するのかもしれない。したがって、実験３ではヒツジの血漿グルコースおよび Leu 代謝動態に及ぼす尿素および糖蜜添加稲わら(RSUM 飼料)給与の影響を測定した。ヒツジ４頭が１期 21 日のクロスオーバー法にしたがって MH 飼料あるいは RSUM 飼料を給与された。それぞれの飼料の 21 日目に同位元素希釈法の primed-continuous infusion 法を実施した。飼料給与量は維持代謝エネルギー(ME)量とし、MH 飼料が混播牧草 57.8 $g/kg^{0.75}/d$、RSUM 飼料が稲わら 59.7 $g/kg^{0.75}/d$、尿素 0.84 $g/kg^{0.75}/d$、糖蜜 7.6 $g/kg^{0.75}/d$ とした。N 摂取量は、MH 飼料に比べて RSUM 飼料がかなり低かったが($P < 0.05$)、N 消化率は両飼料で類似していた。ルーメン NH_3 およびプロピオン酸濃度は MH 飼料よりも RSUM 飼料が高かった($P < 0.05$)。血漿グルコース代謝回転速度は飼料間に差がなかったが、数値的には MH 飼料と比べて RSUM 飼料がやや高かった。血漿 LeuTR、WBPS および WBPD は RSUM 飼料と MH 飼料とで同等であった。

実験４

実験２および実験３から窒素化合物とエネルギー基質を添加した稲わらは混播牧草と同等の成績が得られることを明らかにした。サイレージ化した窒素化合物・エネルギー基質を添加した稲わらは発酵を通じて血漿栄養素代謝に影響を与えることが期待される。したがって、実験４ではヒツジの血漿酢酸、グルコースおよび Leu 代謝に及ぼす尿素と糖蜜添加稲わらサイレージ(RSUMS 飼料)給与の影響を評価するため、[1-

^{13}C]酢酸 Na、[U-^{13}C]グルコース、 [1-^{13}C]Leu の同位元素希釈法を実施した。ヒツジ４頭が１期 21 日のクロスオーバー法にしたがって MH 飼料と RSUMS 飼料に割り分けられた。飼料給与量は現物で RSUMS 飼料が尿素・糖蜜添加稲わらサイレージ 109.9 g/kg$^{0.75}$/d、MH 飼料 57.8 g/kg$^{0.75}$/d とした。粗タンパク質給与量は RSUMS 飼料 5.4 g/kg$^{0.75}$/d、MH 飼料が混播牧草 6.4 g/kg$^{0.75}$/d であった。N 摂取量は MH 飼料に比べて RSUMS 飼料が少なかったが($P < 0.05$)、N 消化率は高かった($P < 0.05$)。ルーメン総 VFA および酢酸濃度は飼料間で差がなく、プロピオン酸濃度は MH 飼料よりも RSUMS 飼料が高い傾向を示した($P = 0.07$)。血漿酢酸およびグルコース代謝回転速度は飼料間に差がなかった。血漿 LeuTR、WBPS および WBPD は両飼料で類似していた。本研究の結果から、尿素と糖蜜を添加した稲わらサイレージは混播牧草と同様に反芻家畜の生産向上のため使用し得ることが示された。

結論

窒素化合物とエネルギー基質を添加した未処理およびサイレージ処理の稲わらは血漿酢酸、グルコースおよびタンパク質代謝に関して混播牧草に匹敵する飼料であると結論される。

将来的な意義

本研究の結果から、稲わらは反芻家畜の飼料として最も重要な副産物の１つであることが示された。稲わらの使用は世界的な飼料不足問題を解決する有望な手段であり、家畜飼養を通じて稲わらを人の栄養に再利用できる優れた技術である。稲わらの効率的利用は家畜生産にとって有益である。したがって、容易に供給可能なタンパク質とエネルギー基質を添加した未処理およびサイレージ処理した稲わらは反芻家畜の生産を向上させ、飼料コストを抑制し、持続可能な地球環境を創出するために広く普及されるべきである。

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