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Original Article

Effects of Replacing Concentrate with Soybean Curd Residue Silage on Ruminal Characteristics, Plasma Leucine and Glucose Turnover Rates in Sheep

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Abstract

The effects of replacing concentrate with soybean curd residue silage on the plasma leucine (Leu) and glucose turnover rates (LeuTR and GluTR) of sheep were evaluated using an isotope dilution method of [1-^{13}C]Leu and [U-^{13}C]glucose. The ruminal characteristics and nitrogen (N) balance of the sheep were also determined. The sheep were fed two diets in a crossover experiment for 21 days. The first diet consisted of 80% mixed hay and 20% commercial concentrate (CON diet), and the second diet consisted of 80% mixed hay and 20% soybean curd residue silage (SCRS diet), on a dry matter basis for both diets. The primed-continuous infusion method of [1-^{13}C]Leu and [U-^{13}C]glucose was performed on day 21. The N absorption, N retention, and N digestibility did not differ between the diets. Furthermore, the ruminal total volatile fatty acid concentration did not differ, but the molar concentration of propionate was significantly higher (P=0.02) in the sheep fed the SCRS diet than the CON diet. The plasma LeuTR and GluTR were comparable between the diets. The results showed that soybean curd residue silage could be comparable with commercial concentrate on ruminal fermentation as well as on the plasma Leu and glucose kinetics. Thus, soybean curd residue silage could be used to replace commercial concentrate in the diet of sheep.

Key words: food by-product, glucose kinetic, isotope dilution, protein kinetic, wether

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Introduction

Economic and environmental concerns prompt investigation into the possibility of using the organic resources generated by the food industry as alternative animal feeds. Soybean curd residue (SCR), a by-product of tofu, soymilk and soy protein manufacturing, is high in protein content; however, because SCR is considered an industrial waste, it is often incinerated due to its rapid rate of deterioration. Ensiling SCR (soybean curd residue silage; SCRS) could prolong its shelf life and increased its nutrient composition and digestibility (Cho *et al*., 2007). An *in vitro* study on the nutritional properties of SCRS showed that the fermentation process also enhanced antioxidant activity (Rashad *et al*., 2001), and previous studies (Oltjen & Putnam, 1966; Clifford & Tillman, 1968; Prior, 1976; Knaus *et al*., 2002) have reported increased nitrogen (N) absorption and N retention when soy protein-based diets were fed to steers and sheep. Inclusion of SCRS in the diet of sheep has been shown to increase the glucogenic propionate concentration in the rumen without any negative effects on ruminal fermentation (Xu *et al*., 2001). Nevertheless, research regarding the use of SCRS as ruminant feed on the N balance and the kinetics of plasma amino acid (AA) and glucose has been limited. Thus, additional studies should be needed to promote the utilization of this by-product as ruminant feed.

We hypothesized that SCRS can replace commercial concentrate in the diet of sheep without any adverse effects on whole-body N and glucose metabolism due to its high soluble N and fermentable carbohydrate contents. Improved utilization of residue generated by the food industry such as SCRS could reduce feed cost and environmental problem. The objective of this study was to determine the effects of replacing a commercial concentrate with SCRS on the plasma leucine (Leu) and glucose turnover rates (LeuTR and GluTR), using an isotope-dilution method with [1-^{13}C]Leu and [U-^{13}C]glucose. The N balance, ruminal characteristics and blood metabolites were also analyzed.

Materials and Methods

Animals and diets

All of the animal handling, including blood sampling, was conducted according to the guidelines established by the Animal Care Committee of Iwate University. Four crossbred (Suffolk x Corriedale) wether, all of approximately 4 years of age and initially of 54 ± 4 kg of body weight were used. This experiment used a crossover design: a 21-day period that consisted of 14 days of dietary adaptation and 7 days of sample collection. Throughout the adaptation period, the sheep were kept in individual pens and fed two dietary treatments. The first diet consisted of 80% mixed hay (orchardgrass and reed canarygrass) and 20% commercial concentrate (CON diet), and the second diet consisted of 80% mixed hay and 20% soybean curd residue silage (SCRS diet), on a dry matter (DM) basis. The SCRS contained 15% beet pulp. The chemical composition of mixed hay, SCRS and commercial concentrate are listed in Table 1. The diets were estimated to be isonitrogenous and the metabolizable energy intakes assumed from the National Research Council (1985) were slightly above the maintenance level for both diets, as shown in Table 2. The sheep were fed twice daily at 08.30 and 20.30 hours and had *ad libitum* access to water. The SCRS was purchased from a food company (Hirakawa Food Co. Ltd., Iwate, Japan), placed into 90 L polyvinyl bags, compacted, and sealed tightly. After 15 days of ensiling, the container was opened and the silage was fed to the animals as reported previously (Amaha et al., 1995).

On day 15, the sheep were moved to individual metabolic cages in a controlled-environment room, with an air temperature of 23°C, a relative humidity of 70%, and lighting from 08.00 to 22.00 hours.

Sample collection

Before starting the feeding experiment, samples of the feed were collected and dried in a forced-air oven at 60°C for 48 hours to determine the chemical composition and to formulate the dietary treatments. Offered feed and refused feed (if any) were sampled and dried using the same procedure for the chemical analysis.

From day 16 to day 20, urine and feces samples were collected at 24-hour intervals using a

3 mm plastic screen separator. The samples were collected once daily at 10.00 hours. The urine was collected from each sheep in a bucket containing 50 mL of 6 *N* H_2SO_4, and the volume was recorded; 50 mL of the urine was stored at -30°C until analysis. Feces samples were dried in a forced-air oven at 60°C for 48 hours and were then passed through a 1 mm mesh and stored at room temperature until analysis.

Table 1: Chemical compositions of the mixed hay, soybean curd residue silage and commercial concentrate

	Mixed hay	Soybean curd residue silage[a]	Commercial concentrate[b]
DM (g/kg)	910	309	880
CP (g/kg DM)	118	183	127
EE (g/kg DM)	30	110	38
NDF (g/kg DM)	680	150	207
ME (Mcal/kg DM)	1.97	3.32[c]	2.68

[a]Soybean curd residue silage contained 15% beet pulp (Hirakawa Food Co. Ltd., Japan)
[b]Commercial concentrate: formula feed ("α-Beef" made by Chubu Feed Co. Ltd., Japan)
[c]Calculated as the proportion of soybean curd residue and beet pulp (85:15) in the silage according to MAFF (1995).
DM: dry matter
CP: crude protein
EE: ether extract
NDF: neutral detergent fiber
ME: metabolizable energy

Table 2: Diet formulation and intakes of crude protein (CP) and metabolizable energy (ME) of the dietary treatments

	Treatment[a]	
	CON diet	SCRS diet
Mixed hay ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	52.6	48.5
Commercial concentrate[b] ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	13.2	0
Soybean curd residue silage ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	0	12.1
CP intake ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	7.9	7.9
ME intake[b] ($Mcal \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	139	135

[a]Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is supplemented with either commercial concentrate (CON diet) or soybean curd residue silage (SCRS diet) at a ratio of 80:20 on dry matter basis.

Ruminal fluid (50 mL) was collected on day 20 at 0 (before feeding), 3 and 6 hours after the morning feeding via a stomach tube. The pH of the ruminal fluid was immediately measured using a pH meter (HM-10P, Toa Electronics Ltd., Japan) after collecting each sample. The liquid fraction of the ruminal fluid was separated by centrifuging at 8,000 x *g* for 10 minutes at 4°C. A 5 mL aliquot of the liquid fraction was used to measure the ruminal VFA content, and 1 mL of the liquid fraction was mixed with 1 mL of a 0.1 mol/L HCl for the ruminal ammonia N (NH_3-N) analysis. All of the samples were stored at -30°C until further analysis.

Measurements of amino acid and glucose kinetics

On day 21 of each dietary treatment, a primed-continuous infusion [1-^{13}C]Leu and [U-^{13}C]glucose method was conducted to determine the plasma Leu and glucose kinetics simultaneously over a period of 4 hours, between 3 and 7 hours after the morning feeding. Polyvinyl catheters were placed in both jugular veins in the morning and filled with

a sterile solution of 3.8% (w/v) trisodium citrate. A mixed saline solution of 7.2 μmol/kg $BW^{0.75}$ [1-^{13}C]Leu (L-leucine 1-^{13}C, 99 atom% excess ^{13}C; Cambridge Isotope Laboratories, USA) and 3.0 μmol/kg $BW^{0.75}$ [U-^{13}C]glucose (D-glucose-$^{13}C_6$, 99 atom% excess ^{13}C; Cambridge Isotope Laboratories, USA) was injected into the right jugular catheter as a priming dose. This solution was then continuously infused through the same catheter using a multichannel peristaltic pump (AC-2120, Atto, Japan) at rates of 7.2 and 3.0 μmol · kg $BW^{-0.75}$· h^{-1} for [1-^{13}C]Leu and [U-^{13}C]glucose, respectively. The infusion rate of the tracer solution was recorded every 30 minutes throughout the infusion period. Blood samples (6 mL) were collected from the left jugular catheter immediately before the priming injection and at 30-minutes interval during the last 2 hours of isotope infusion (5 to 7 hours after feeding). The blood samples were placed in heparinized tubes and stored in ice. The plasma from the blood sample was separated by centrifugation at 8,000 x *g* for 10 minutes at 4°C and then stored at -30°C until further analysis.

Sample analysis

The chemical components of the diets were analyzed according to the Association of Official Analytical Chemists (AOAC, 1990). The N contents in the diets, feces and urine were analyzed using the Kjeldahl method (Kjeltec 2100, Foss, Sweden). The fermentation products of the silage were analyzed using cold-water extracts (Cai *et al*., 1999). The pH values of the silage and ruminal fluid were measured using a pH meter (HM-10P, Toa Electronics Ltd., Japan). The lactic acid concentration of the silage was measured by the colorimetric method (Taylor, 1996), and the absorbance was measured using a spectrophotometer (U-1000, Hitachi, Japan) at 570 nm. The molar VFA concentrations of the silage and ruminal fluid were determined using gas chromatography (HP-5890, Hewlett Packard, USA) after steam distillation. The volatile basic N (VBN) content of the silage was determined based on the steam distillation method described by Dhaouadi *et al*. (2007). To assess the quality of silage, we calculated the Flieg point from the DM and pH values (Yilmaz *et al*., 2009) and the V-score from the VBN/total N and VFA concentrations (Takahashi *et al*., 2005).

The concentrations of plasma Leu and α-ketoisocaproic acid (α-KIC) and enrichments of the plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC were determined by gas chromatography mass spectrometry (QP-2010, Shimadzu, Japan) with selected ion monitoring according to the procedures of Rocchiccioli *et al*. (1981) and Calder & Smith (1988). The plasma [U-^{13}C]glucose enrichment was measured using the methods of Tserng & Kalhan (1983) with slight modifications described by Fujita *et al*. (2006). The plasma glucose concentration was measured by the glucose oxidase method (Huggett & Nixon, 1957). The plasma free AA and urea concentrations were determined using an automated AA analyzer (JLC-500/V, JEOL, Japan). The plasma non-esterified fatty acid (NEFA) concentrations were determined enzymatically using a commercial diagnostic kit (NEFA-C test, Wako, Japan).

Calculations

The turnover rates of plasma Leu and glucose (LeuTR and GluTR) were calculated according to the equation described by Wolfe (1984):

$$TR\ (mmol \cdot kg\ BW^{-0.75} \cdot h^{-1}) = I \times (1 / E - 1)$$

where I represents the infusion rates of [1-^{13}C]Leu and [U-^{13}C]glucose and E represents the isotope enrichments of the plasma α-[1-^{13}C]KIC, metabolite of [1-^{13}C]Leu, and plasma [U-^{13}C]glucose at the steady state.

Statistical Analysis

The mean values and standard errors of the mean (SEM) were calculated for all of the data. All of the data were analyzed using the MIXED procedure in SAS (1996). The fixed effects in the model were the period, diet, and the period x diet interaction, and the random effect was the sheep. The results were considered significant for $P<0.05$, and the tendency was defined as $0.05 \leq P < 0.10$. Repeated statements were used for the time course of the ruminal characteristics, and the difference of the least square means using Tukey's adjustment was used ($P<0.05$).

Results

Silage quality and N balance

The fermentative characteristics of the SCRS are shown in Table 3. The pH value was 4.14, and organic acids, such as lactic acid, acetate, propionate and butyrate, were detected. The effects of replacing commercial concentrate with SCRS on the N balance are presented in Table 4. Although both diets were estimated to be isonitrogenous, the N intake determined at the end of the experiment tended (P=0.05) to be higher for the SCRS diet than the CON diet. The fecal N excretion did not differ (P=0.17), but the urinary N excretion tended to be higher (P=0.05) for sheep fed the SCRS diet than those fed the CON diet. Both groups of sheep retained similar amounts of N (P=0.42), and the N absorption and digestibility did not differ (P=0.11 and P=0.14, respectively) between the two diets.

Table 3: Fermentative characteristics of the soybean curd residue silage

Item	Amount
Moisture (g/kg)	690
pH	4.14
Lactic acid (g/kg FM[a])	40
Acetate (g/kg FM)	1.1
Propionate (g/kg FM)	0.03
Butyrate (g/kg FM)	0.003
VBN[b] (g/kg total N)	68
Flieg point	100
V-score	98

[a]FM: fresh matter
[b]VBN: volatile basic nitrogen

Table 4: Effects of feeding soybean curd residue silage on the nitrogen (N) balance in sheep[a]

Items	Treatment[c]		SEM[b]	*P* value
	CON diet	SCRS diet		
N intake ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	1.267	1.278	0.005	0.05
Fecal N ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	0.48	0.45	0.03	0.17
Urinary N ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	0.48	0.55	0.07	0.05
N absorption ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	0.79	0.83	0.01	0.11
N retention ($g \cdot kg\ BW^{-0.75} \cdot d^{-1}$)	0.31	0.28	0.02	0.42
N digestibility (%)	62.5	65.0	1.0	0.14

[a]Values represent the means for n = 4.
[b]SEM: standard error of the mean.
[c]Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is supplemented with either commercial concentrate (CON diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on dry matter basis.

Ruminal characteristics

The observed changes in the ruminal characteristics during the sampling period are provided in Figure 1. The effects of the two diets on the ruminal pH, NH_3-N and VFA concentrations, represented as the mean value of four sheep in three sampling periods (0, 3, and 6 hours after feeding), are shown in Table 5. No interaction was observed between the dietary treatment and the sampling time for the ruminal characteristics (P>0.10). The sheep that consumed the SCRS tended (P=0.05) to have a higher ruminal pH than those fed the CON diet. The pH values under both treatments were above 6.8 prior to feeding, and the values dropped to approximately 6.7 at 3 hours after feeding.

Table 5: Effects of feeding soybean curd residue silage on the ruminal characteristics of sheep[a]

	Treatment[c]			*P* value		
Item	**CON diet**	**SCRS diet**	**SEM[b]**	**Treatment**	**Time**	**Treatment x Time**
pH	6.69	6.87	0.03	0.05	0.008	0.19
NH_3-N (mmol/L)	11.2	11.9	0.5	0.22	0.002	0.73
Total VFA (mmol/L)	87.9	93.4	1.5	0.13	0.002	0.68
Individual VFA concentrations (mmol/L)						
Acetate	61.7	64.9	0.4	0.26	0.007	0.88
Propionate	15.9	19.2	0.8	0.02	<0.001	0.18
Isobutyrate	0.7	0.8	0.1	0.05	0.01	0.56
Butyrate	8.3	6.8	0.4	0.02	0.07	0.68
Isovalerate	0.8	1.1	0.1	0.06	0.007	0.76
Valerate	0.5	0.6	0.04	0.02	0.001	0.29
Acetate:propionate ratio	3.9	3.4	0.2	0.03	0.008	0.63

[a]Values represent the means for n = 4.
[b]SEM: standard error of the mean.
[c]Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is supplemented with either commercial concentrate (CON diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on dry matter basis.
NH_3-N: ammonia nitrogen; VFA: volatile fatty acid

Comparable NH_3-N concentrations were found for both groups (P=0.22); the NH_3-N concentrations were increased at 3 hours after feeding and eventually returned to the previous values under both dietary treatments.

The total VFA concentration in the rumen did not differ significantly (P=0.13) between the two diets. The acetate concentrations were similar for the two diets, but the propionate concentration was higher (P=0.02) in the sheep fed the SCRS than the CON diet, resulting in a lower acetate:propionate ratio (P=0.03) for the SCRS than CON diet. The butyrate concentration was lower (P=0.02) for the SCRS diet compared to the CON diet, whereas the valerate concentration was higher (P=0.02) and other branched-chain VFA (isobutyrate and isovalerate) concentrations tended to be higher (P=0.05 and P=0.06, respectively) under the SCRS treatment. The total VFA concentration and the concentrations of acetate, propionate, butyrate, and valerate were increased at 3 hours after feeding and eventually returned toward previous values. The isobutyrate and isovalerate concentrations were gradually decreased at 6 hours after feeding.

Pre-isotope infusion plasma concentration of blood metabolites

The plasma AA, urea and NEFA concentrations determined at the pre-isotope infusion period at 3 hours after feeding are presented in Table 6.

Table 6: Effects of feeding soybean curd residue silage on the plasma amino acid, urea and non-esterified fatty acid concentrations during pre-isotope infusion in sheep[a]

Item	Treatment[c]		SEM[b]	*P* value
	CON diet	SCRS diet		
Essential AA (μmol/L)				
Threonine	232	235	13	0.83
Valine	290	259	13	0.14
Methionine	36	34	1.3	0.07
Isoleucine	105	89	19	0.02
Leucine	158	130	7	0.03
Phenylalanine	69	60	2	0.03
Histidine	65	57	2	0.05
Lysine	118	100	7	0.13
Non-essential AA (μmol/L)				
Serine	151	131	16	0.34
Asparagine	58	48	5	0.19
Glutamic acid	52	58	4	0.28
Glutamine	390	336	54	0.11
Glycine	584	523	40	0.27
Alanine	197	173	15	0.25
Tyrosine	98	86	3	0.04
Arginine	133	118	4	0.07
Proline	129	107	8	0.09
Urea, mmol/L	6.2	7.8	0.2	0.02
NEFA, mEq/L	0.10	0.13	0.03	0.38

[a]Values represent the means for n = 4.
[b]SEM: standard error of the mean.
[c]Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is supplemented with either commercial concentrate (CON diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on dry matter basis.
AA: amino acid
NEFA: non-esterified fatty acid

The plasma isoleucine, Leu, phenylalanine, and tyrosine concentrations were lower (P<0.05) and the plasma methionine, histidine, arginine, and proline concentrations tended to be lower (P<0.10)

in the sheep fed the SCRS than CON diet; the concentrations of other AA were comparable between the diets. The plasma urea concentration was higher (P=0.02) in the sheep fed the SCRS than CON diet, and the plasma NEFA concentration did not differ (P=0.38) between the diets.

Amino acid and glucose kinetics

The plasma Leu and α-KIC concentrations and plasma α-[1-^{13}C]KIC enrichment were in a steady state during the last 2 hours of isotope infusion (Figure 2). The plasma Leu and α-KIC concentrations did not differ (P=0.18 and P=0.13, respectively) between the diets (Table 7). The sheep fed the SCRS had similar (P=0.74) plasma LeuTR with those of sheep fed the CON diet. The plasma glucose concentration and [U-^{13}C]glucose enrichment were essentially constant during the last 2 hours of the isotope infusion (Figure 3). The plasma glucose concentration and GluTR did not differ (P=0.68 and P=0.27, respectively) between the two diets.

Table 7: Effects of feeding soybean curd residue silage on the plasma leucine (Leu) and glucose kinetics in sheep[a]

Item	Treatment[c]		SEM[b]	*P* value
	CON diet	SCRS diet		
Leu concentration (μmol/L)	135	107	13	0.18
α-KIC concentration (μmol/L)	12.8	10.3	0.5	0.13
LeuTR (mmol · kg BW$^{-0.75}$ · h^{-1})	0.52	0.51	0.05	0.74
Glucose concentration (mmol/L)	3.34	3.40	0.18	0.68
GluTR (mmol · kg BW$^{-0.75}$ · h^{-1})	2.24	2.14	0.09	0.27

[a]Values represent the means for n = 4.
[b]SEM: standard error of the mean.
[c]Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is supplemented with either commercial concentrate (CON diet) or soybean curd residue silage (SCRS diet) at a ratio of 80:20 on dry matter basis.
α-KIC: α-ketoisocaproic acid
LeuTR: leucine turnover rate
GluTR: glucose turnover rate

Discussion

Soybean curd residue silage quality

The SCRS was well preserved, as indicated by the low pH, high lactic acid content, high Flieg point and V-score. The lactic acid concentration in our SCRS was similar to that found by Amaha *et al*. (1996), but it was higher than that found by Xu *et al*. (2001). During the fermentation process, some hemicellulose and cellulose were broken down into various simple sugars and smaller end products, such as lactic acid, acetate and butyrate (Cao *et al*., 2009). The NDF content in our SCRS (150 g/kg DM) was comparable to the NDF content reported from raw SCR (145 g/kg DM) by O'Toole (1999).

Nitrogen balance

Previous studies (Oltjen & Putnam, 1966; Clifford & Tillman, 1968; Prior, 1976; Knaus *et al*., 2002) have shown that inclusion soy protein in the diet of ruminants enhances the N intake, urinary N excretion, N retention and N absorption. Sano et al. (2009) studied the effect of different N sources supplementation (urea and soybean meal) in the isonitrogenous design and found a large urinary N excretion difference between treatments, indicating a change in N retention. In the present study, replacing the commercial concentrate with SCRS did not affect the amount of N absorbed and retained, whereas it did slightly affect the urinary N excretion. Hence, our findings demonstrate the feasibility of replacing commercial concentrate with soybean curd residue silage to maintain a positive N balance.

Ruminal characteristics

The ruminal pH values of both groups of sheep were above 6.5 for all of the sampling times, and this is the optimal pH for normal ruminal fermentation.

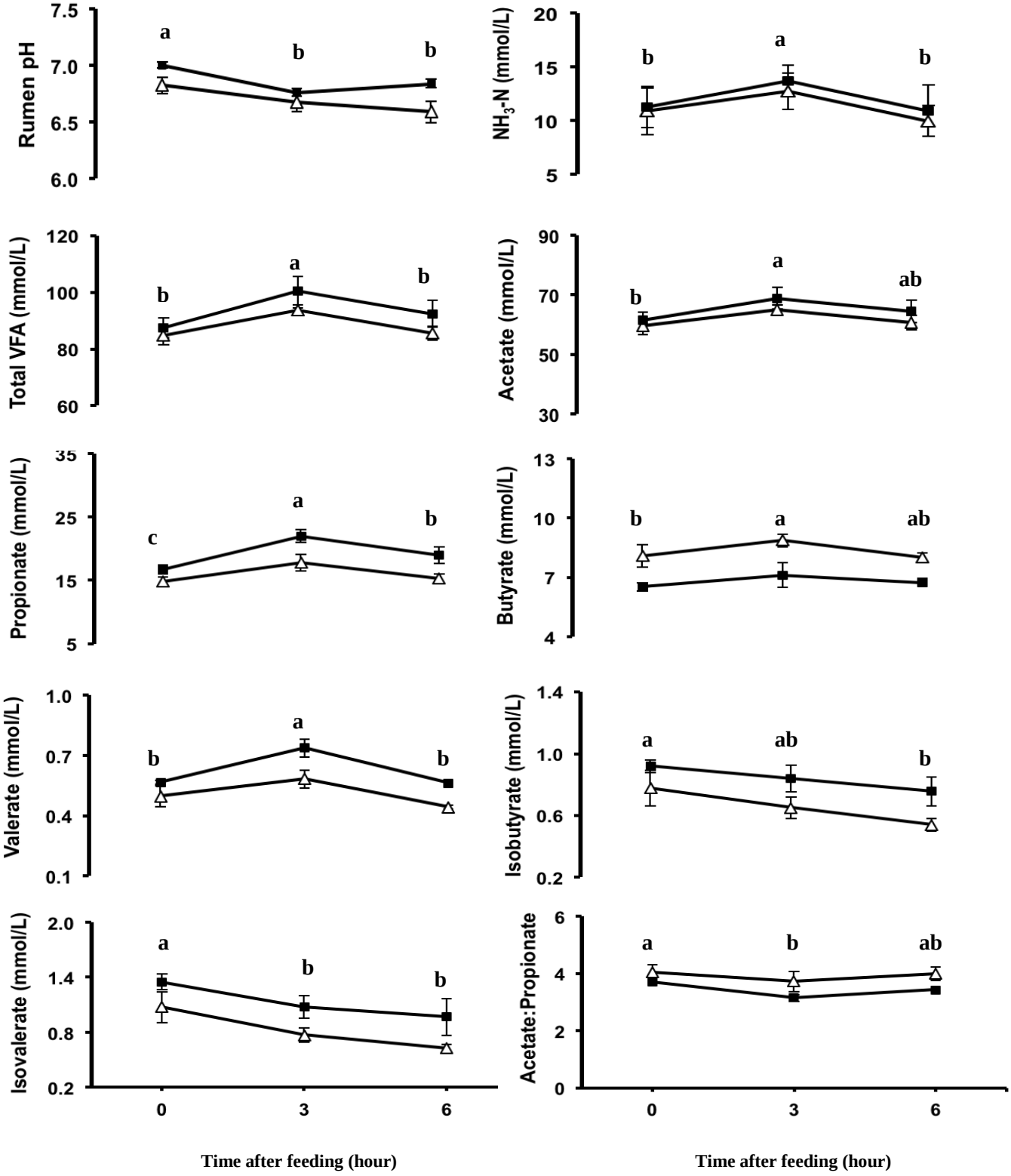

Fig. 1: Time-course changes of ruminal characteristics in sheep fed the SCRS diet (■) and the CON diet (Δ). SCRS diet, mixed hay supplemented with soybean curd residue silage; CON diet, mixed hay supplemented with commercial concentrate. A ratio of 80:20 (dry matter basis) was used for both diets. Ruminal fluid samples were collected at 0 (before feeding), 3 and 6 hours after morning feeding via a stomach tube. Values are expressed as mean ± SEM for n = 4. Different letters (a, b, c) indicate significant different (P<0.05) between time after feeding within each treatment.

Thus, replacing the commercial concentrate with the SCRS did not impair ruminal fermentation; a similar response observed by Xu et al. (2001). The SCRS and CON diets in the present study were approximately isonitrogenous and isoenergetic; thus, similar N compounds should be available for NH_3-N production in the rumen. This finding is consistent with the results of Ipharraguerre *et al*. (2005) who reported that the concentration of NH_3-N in the rumen fluid only affected by the level of dietary CP intakes.

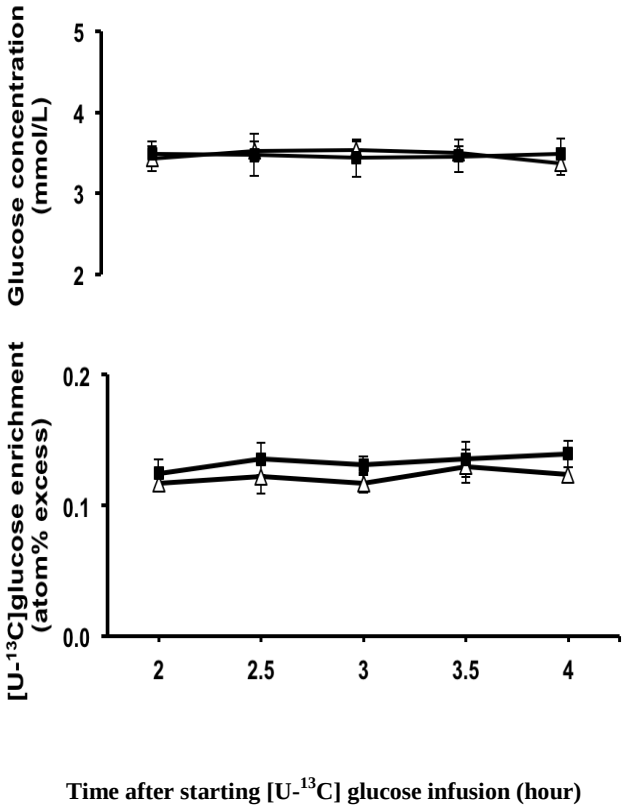

Fig. 2: Time course changes of plasma leucine (Leu) and α-ketoisocaproic acid (α-KIC) concentrations, plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichments during the last 2 hours of isotope infusion in sheep fed the SCRS diet (■) and the CON diet (Δ). SCRS diet, mixed hay supplemented with soybean curd residue silage; CON diet, mixed hay supplemented with commercial concentrate. A ratio of 80:20 (dry matter basis) was used for both diets. Values are expressed as mean ± SEM for n = 4.

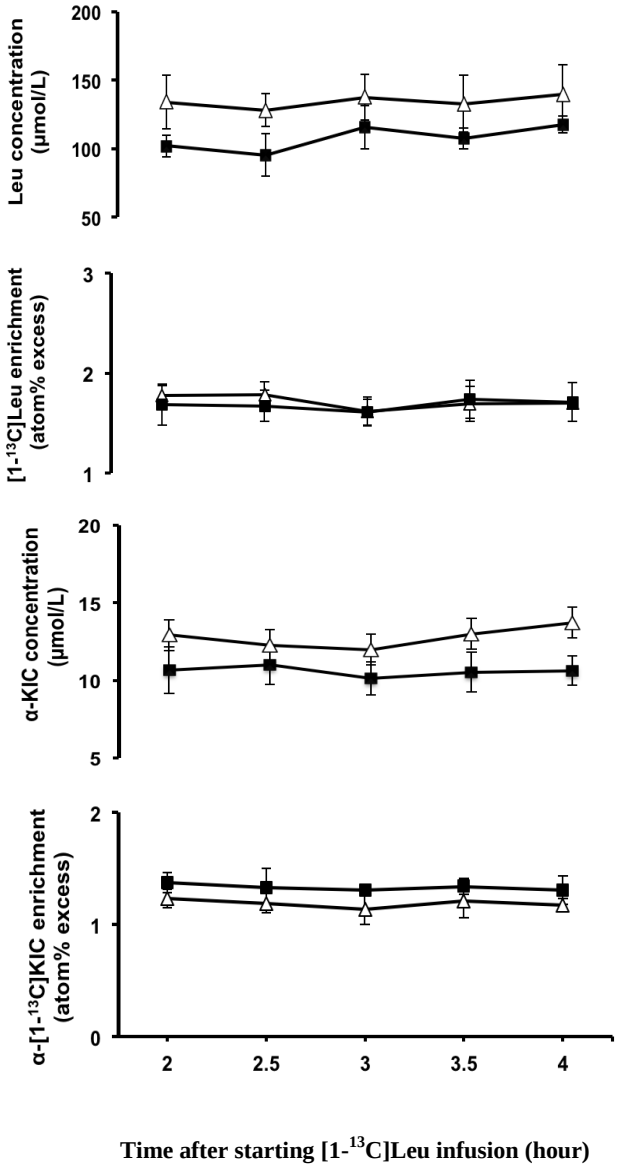

Fig. 2: Time course changes of plasma leucine (Leu) and α-ketoisocaproic acid (α-KIC) concentrations, plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichments during the last 2 hours of isotope infusion in sheep fed the SCRS diet (■) and the CON diet (Δ). SCRS diet, mixed hay supplemented with soybean curd residue silage; CON diet, mixed hay supplemented with commercial concentrate. A ratio of 80:20 (dry matter basis) was used for both diets. Values are expressed as mean ± SEM for n = 4.

Volatile fatty acids are produced in the rumen as the end product of microbial fermentation, and their production largely depends on the type of carbohydrate ingested (Djikstra, 1994). The large increment in the ruminal propionate concentration in the sheep fed the SCRS diet could be attributed to the highly soluble carbohydrate and starch contents (Yang, 2005). Although the NDF concentration in a ruminant diet is positively correlated with the amount of VFA produced in the rumen (Beauchemin, 1991), the lower NDF content for the SCRS (150 g/kg DM) compared to the commercial concentrate (207 g/kg DM) did not alter the total VFA concentration in the rumen. Replacing the commercial concentrate with the SCRS did not significantly influence the ruminal acetate concentration, indicating similar efficiencies of energy-source production for both diets.

Pre-isotope infusion plasma concentration of blood metabolites

The primary source of dietary protein (Schelling *et al*., 1967) affects the plasma AA concentrations in sheep. For the different protein sources in the present study, the lower concentrations of certain plasma AAs in the sheep fed the SCRS diet might not be indicative of the total AA supply because the protein supply in both treatments was similar and sufficient. Thus, the observed lower AA levels were most likely due to the limiting AAs in the diet (Lapiere & Lobley, 2001), which, in soybean-based diets, are sulfur-containing AAs (methionine and cysteine) (Ma *et al*., 1996). Moreover, Lapierre and Lobley (2001) suggested that the concentration of plasma AA after feeding varied depend on the rate of digestion and AA catabolism of the dietary protein source. Furthermore, these authors noted that approximately 46% of AA from a highly degradable type of protein such as silage did not appear in the peripheral vein, whereas for a concentrate the amount varied between 23% and 24%. Although the sheep fed the SCRS diet in our study exhibited lower concentrations of certain plasma AAs, replacing the commercial concentrate with the SCRS did not induce an AA imbalance, based on the positive N balance and similar N retention for both of the diets.

The use of different protein sources affected the plasma urea concentration, which is a metabolic end product of protein catabolism or the product of soluble protein fermentation in the rumen. Although the ruminal NH_3-N concentrations within the sampling periods (0, 3, and 6 hours after feeding) did not differ significantly between the two diets, the high plasma urea concentration for the SCRS diet, reflected by the rapid degradation of N compounds and the rapid absorption of NH_3-N through the rumen, resulted in an increased level of plasma urea. Furthermore, the N excretion via the urine tended to increase over time. Similarly, Leibholz and Cook (1967) showed that elevated plasma urea concentrations increased the N excretion rate via the urine and the rate of return of N to the rumen as urea in the saliva in sheep fed alfalfa hay and concentrate.

The circulating plasma NEFA concentration was markedly related to the nutritional status. The plasma NEFA concentration increases with high rates of lipolysis, such as occurs with fasting, negative energy balance or stress (Trenkle & Kuhlemeier, 1966). Hence, the comparable plasma NEFA concentrations indicated comparable nutritional status between sheep fed the SCRS and CON diet.

Amino acid and glucose kinetics

In the present study, both plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichments were determined, but only plasma α-[1-^{13}C]KIC enrichment is discussed because it represents an accurate index of the intra- and extra-cellular amino acid exchange (Magni *et al*., 1994). The plasma LeuTR observed in our study were comparable to previous studies using single-isotope infusion of [1-^{13}C]Leu to determine protein kinetics in sheep (Sano *et al*., 2004; 2009); similarly, the plasma GluTR observed in our study were comparable to those from previous study on glucose kinetic in sheep determined using single-isotope infusion of [U-^{13}C] glucose (Sano et *al*., 2000). These results indicate that the double isotope used in our experiment did not alter either the plasma Leu or

glucose kinetics of the sheep. The use of different protein sources resulted in varying plasma AA concentrations, which could influence the turnover rate of proteins (Nielsen *et al*. 1993). Although reports on the AA kinetics of soybean-based diets in ruminants are limited, the results from other studies in pigs (Deutz *et al.*, 1998) and humans (Bos *et al*., 2003) showed that the AAs from soybean-based diets are rapidly digested and absorbed, indicating an enhanced AA catabolism. The low concentration of plasma AAs in the sheep fed the SCRS diet could have been caused by the utilization of AAs as an energy substrate to support protein turnover within the tissues; thus, the plasma LeuTR measured were comparable between the two diets. In addition, the dietary treatments were approximately isoenergetic and isonitrogenous, which could have contributed to the comparable plasma LeuTR for the two diets because the plasma LeuTR was positively correlated with the dietary protein and metabolizable energy intake in sheep (Liu et *al*., 1995) and cows (Lapierre *et al*., 2002). Similarly, Sano *et al*. (2009) reported comparable plasma LeuTR for different protein sources (urea and soybean meal) in sheep fed the isoenergetic and isonitrogenous diets.

The GluTR is influenced by several factors, including the energy intake and supply of gluconeogenetic substrate to the liver (Ortiques-Marty *et al*., 2003). The propionate produced in the rumen is a major glucogenic substrate and a precursor of de novo glucose synthesis (Herbein *et al*., 1978). In the present study, although the glucogenic propionate concentration in the rumen was higher for the SCRS than CON diet, the plasma GluTR were similar for both of the diets. In accordance with this result, an increasing supply of glucogenic propionate did not systematically increase plasma GluTR in steers (Seal & Parker, 1994) and sheep (Linington *et al*., 1998). Herbein *et al*. (1978) and Seal *et al*. (1992) pointed out that similar energy intake resulted in similar plasma GluTR in sheep and growing steers. Thus, the isoenergetic diets in the present study did not significantly influence the plasma GluTR. The comparable results for the plasma Leu and glucose kinetics indicated similar nutritive value for the two diets. Similar plasma Leu and glucose kinetic responses could be achieved by replacing commercial concentrate with SCRS in the same amount of metabolizable energy intake.

In conclusion, replacing commercial concentrate with SCRS in the diet of sheep did not impair ruminal fermentation, and enhanced the glucogenic propionate concentration. The inclusion of SCRS in the diets of sheep could ensure a positive N balance and resulted in similar plasma AA and glucose kinetics with commercial concentrate when formulated in the same energy intake. Thus, feed cost can be reduced by replacing commercial concentrate with soybean curd residue silage. Furthermore, technological developments in the use of residue and by-products will help to improve the feed self-sufficiency rates and make animal agriculture more sustainable.

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