Studies on the effects of feeding soybean curd residue silage on whole body nutrient metabolism in sheep

2012. 9

The United Graduate School of Agricultural Sciences
Graduate School of Iwate University
Science of Bioproduction

(Iwate University)

Dian Wahyu Harjanti

Studies on the effects of feeding soybean curd residue silage on whole body nutrient metabolism in sheep

A Dissertation

Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

September, 2012

by

Dian Wahyu Harjanti

Under the supervision of

Professor Dr. Hiroaki SANO

The United Graduate School of Agricultural Sciences
Science of Bioproduction
Animal Nutrition and Physiology
Iwate University

Contents

Chapter-4

Chapter-5

Chapter-6

Dedication

I dedicate this thesis to my parents, husband, brother and son for their love, continuing prayers, support, encouragement and patient throughout my time in Japan

Dian Wahyu Harjanti

September 2012

Acknowledgements

In the name of Allah, Most Gracious, Most Merciful.
First of all, to Allah who is the Most Loving and the Gracious, for giving me the mercy and endowed me with the insight to include this research study to the unlimited ocean of knowledge

I acknowledge with appreciation and feel much delighted to express my heartiest and deep sense of gratitude and sincere appreciation to my supervisor Professor Dr. Sano Hiroaki, for his affectionate supervision, precious guidance, inspiring attitude and compassionate facilitation to make this study successfully accomplished.

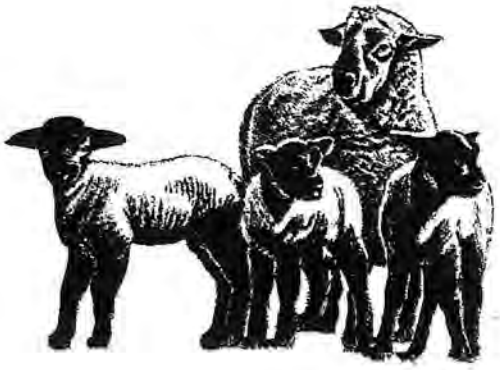
I would like to thank President of Diponegoro University and Ministry of Education and Culture of the Republic of Indonesia for the financial support to my study at Iwate University.

I feel proud to express my heartfelt gratitude to Professor Dr. Joelal Achmadi for his generous guidance, encouragement, support, commendable suggestion and opportunity to pursue my study at Iwate University. Many thanks are extended to the staff of Faculty of Animal Science and Agriculture, Diponegoro University, particularly to Professor Dr. Umiyati Atmo Marsono and Professor Dr. Agung Purnomoadi for their valuable support and kind advice.

I feel proud to express my heartfelt gratitude to the committee members: Professor Dr. Kita Kazumi at Department of Animal Sciences, Faculty of Agriculture, Iwate University; Professor Dr. Horiguchi Kenichi at Department of Animal Sciences, Faculty of Agriculture, Yamagata University; and Professor Dr. Matsuzaki Masatoshi at Department of Bioproduction, Faculty of Agriculture and Life Science, Hirosaki University for their constructive criticisms, valuable comment and kind help in completing this thesis. I am also glad to express my deep sense of gratitude to Professor Dr. Nagasawa Takashi who has given me opportunities to use automated liquid gas chromatography.

My deepest gratitude to Dr. Mohammad Al-Mamun and my research partners: Sugawara Yurie, Komatsuda Satochi, Kawai Hiromi, Sekiguchi Natsumi and Bi Xue and for all of members of Laboratory of Animal Nutrition and Physiology for their warmest friendship, support, kind help and corporation during research work. With them, I always feel like home. Thank also goes to Hirakawa Food Co. Ltd
in Iwate Prefecture for providing soybean curd residue silage during experiment.

And endless love and appreciation go to my husband, son, parents and brother for their continuous inspiration, encouragement and support in pursuing this study possible.



"To be a scientist you need curiosity, self-confidence and the great virtue of keeping the enthusiasm when failing"

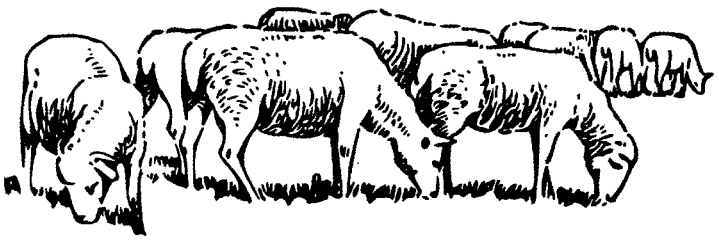
"To pursue a PhD abroad, indeed is a life experience, but the most rewarding is not only the degree itself but also the people that you meet on the way"

Dian Harjanti
Japan2012

Abbreviations

AA	Amino acid
BW	Body weight
CP	Crude protein
DM	Dry matter
EE	Ether extract
FM	Fresh matter
GC-MS	Gas chromatography-mass spectrometry
GluTR	Glucose turnover rate
α-KIC	α-ketoisocaproic acid
KVA	Ketovaleric acid
Leu	Leucine
LeuTR	Leucine turnover rate
ME	Metabolizable energy
MTBSTFA	*N*-methyl-*N*-t-butyl-dimethylsilyltrifluoroacetamide
N	Nitrogen
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
NFC	Non-fiber carbohydrate
NH_3	Ammonia
NPN	Non-protein nitrogen
RDP	Ruminally degradable protein
RUP	Ruminally undegradable protein

SCR	Soybean curd residue
SCRS	Soybean curd residue silage
SSA	Sulfosalicyclic acid
VBN	Volatile basic nitrogen
VFA	Volatile fatty acid
WBPD	Whole body protein degradation
WBPF	Whole body protein flux
WBPS	Whole body protein synthesis
WSC	Water soluble carbohydrate



Chapter 1

General Introduction

Recent issues in the global livestock industry

Animal products provide one-sixth of all human food energy and more than one-third of human protein consumption globally (Bradford, 1999). Projections for the future suggest that there will be large increases in the per capita demand for animal food products in developed and developing countries. With increases in livestock production, the demand for livestock feed will also increase, and there will be more competition for grains and crops for use as human food and animal feed. In Japan, the domestic supply of roughage accounts for less than 80% of the amount used, and domestic concentrate accounts for only 10%. Thus, the total livestock feed self-sufficiency ratio is only 26% (MAFF, 2009). Japan depends heavily on imports for its feed supply; approximately 75% of the total digestive nutrition of the 25,286,000 metric tons of feed consumed in Japan is imported (Sugiura et al., 2009). In addition, there is great political and social pressure to reduce the pollution generated by industrial activities. Consequently, most countries are moving towards a model in which food industrial residues are no longer treated as waste but are considered as potential resources and energy sources. Future efforts should therefore be focused on increasing the domestic feed supply by utilizing currently unused resources and promoting the use of by-products or residues generated by the food industry. Food industry residue may be able to replace imported commercial feeds, and its utilization will reduce the environmental impact of burning or landfilling food wastes. However, choosing safe material for livestock feed is important, particularly in light of increasing concerns about food safety for human consumption. Since the outbreak of bovine spongiform encephalopathy, material containing animal protein has been banned from use as ruminant feed. The European Union and North American regulations against the use of animal protein supplements in ruminant diets and have promoted research into the use of plant protein concentrates (Hasha, 2002;

FDA, 2004). In August 2006, the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) issued guidelines to ensure the safety of non-animal protein material as a ruminant feed (Sugiura et al., 2009).

Soybean curd residue

Soybean is an important food seed with high protein and oil content, and many foodstuffs are made from it. Soybean curd residue is also called okara (Ma et al., 1996), tofu cake (Kakihara et al., 2004) or soy pulp (O'Toole et al., 1999) is the filtrated residue from which soybean milk is separated. It is produced in large quantities in conjunction with the manufacture of soy milk and soybean curd (tofu), especially in Asian areas. During soybean curd production, especially the filtration step (Figure 1.1), up to 30% of the soybean is lost as a waste product (Kim and Lee, 2010). Approximately 700,000 metric tons of soybean curd residue from manufacturing companies are produced annually in Japan (Amaha, 1996; Kajikawa 1996), which suggests that soybean curd residue could be a potential source of low-cost plant protein to be used in animal feed.

Large quantities of soybean curd residue are dumped in landfills or burned, which causes environmental problems (Ohno et al., 1996). Soybean curd residue is treated as industrial waste with little market value because of its short shelf life (O'Toole, 1999). It deteriorates rapidly upon exposure to air because of its low dry matter (DM) and high moisture contents, which vary from 65% to 75% of the fresh matter (FM) (Amaha et al., 1996; Xu et al., 2001; Yang et al., 2005; Rashad et al., 2011). However, many nutrients remain in soybean curd residues after the residues have passed through a filter press. Soybean curd residue generally possesses high nutritive quality because of its degradable protein content, which is high because the soybean

protein molecules are denatured and protease inhibitors are destroyed during the manufacturing process (Kamata et al., 1979). The protein content varies from 18% to 27% DM (Kajikawa, 1996; Ma et al., 1996; O'Toole, 1999; Hirayama et al., 2002; Kakihara et al., 2004). Previously, Ma et al. (1996) studied the isolation and characterization of protein from soybean curd residue. They reported that soybean curd residue has high *in vitro* protein digestibility. Moreover, the essential and non-essential amino acid (AA) profiles in soybean curd residue were comparable to the Food and Agriculture Organization (FAO) scoring pattern, with sulfuric AAs (methionine and cysteine) and tyrosine as the limiting AAs (Ma et al., 1996). Raw soybean curd residue generally contains 12-17% soluble fiber (Chiou et al., 1998; O'Toole, 1999), 11-16.6% ether extract (EE) (Enishi et al., 2004; Ieki et al., 2010), minerals and vitamins (Ikeda and Murakami, 1995; Yokota et al., 1996). Therefore, soybean curd residue is a prospective supplement for farm animal feed.

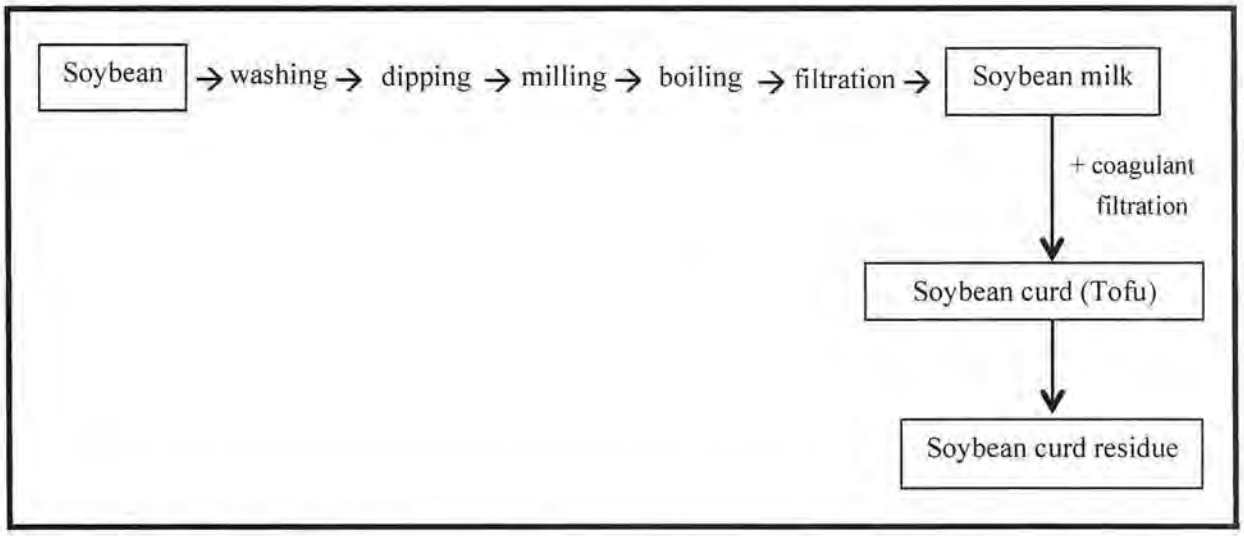


Figure 1.1. Flow chart of soybean curd (tofu) production. The soybeans are soaked in the water and grounded before heated at 95-100°C for 3 minutes. A coagulant consisted of a gypsum powder or a solution of magnesium salt (nigari) is added into the soybean slurry. The free supernatant whey is removed, and then the soybean curd is transferred into perforated boxes and pressed until a coherent block of curd obtained. The soybean curd is next immersed in cold running water for several hours, with the purpose of cooling and leaching out excess coagulant and entrapped whey solids. The soybean curd block is cut into retail-size portions and wrapped for marketing. Adapted from Amaha et al. (1996).

The advantages of ensiling soybean curd residue

Aerobic deterioration begins within half a day during the summer if raw soybean curd residue is left untreated. Once spoilage has begun, the soybean curd residue is less palatable and can cause diarrhea and ketosis. Soybean curd residue manufacturers are mostly located in urban areas, whereas livestock farms tend to be in more remote locations (Amaha et al., 1996). With long-distance transport, the risk or spoilage and transportation costs increase. However, if soybean curd residue can be stored without spoilage for longer period, it does not have to be delivered to farms everyday. Ensilage is the most convenient way to preserve food industrial by-products that contain low DM (Amaha et al., 1996; Yang et al., 2005; Kondo et al., 2006; Cao et al., 2009), such as soybean curd residue. Although considerable biochemical changes occur during fermentation, especially to the carbohydrate and protein balance, the overall DM and energy losses arising from the activities of lactic acid bacteria are low (McDonald, 2011). Moreover, the fermentation process eliminates anti-nutritional properties and may even increase nutrient levels through microbial synthesis (Cho et al., 2007; Kondo et al., 2006). The fermentation process results in highly soluble nitrogenous contents relative to those present in raw material, which may increase the digestibility in animals. During fermentation, extensive degradation of the nitrogenous compound occurs, which produces volatile nitrogen (N) that may adversely affect the ruminal fermentation of the host animal. McDonald et al. (2011) indicated that well-preserved silage should have a volatile basic nitrogen (VBN) content of less than 100 g per total N.

Additionally, the generation of silage from materials with high moisture content, such as soybean curd residue, presents the risk of effluent production during ensiling. Therefore, soybean curd residue is usually mixed with other dry feedstuffs as an effective way to prevent nutrient loss, as suggested by Kajikawa et al. (1996). Wang and

Nishino (2008) compared the fermentative quality of soybean curd residue ensiled alone with that of residue ensiled after mixing with other ingredients. The soybean curd residue silage containing other ingredients had a lower pH (3.49) and higher lactic acid content (2.23%) than soybean curd residue ensiled alone (4.38 and 0.52%, respectively), indicating better fermentation and silage quality for soybean curd residue ensiled with other ingredients than those ensiled alone. Indeed, McDonald et al. (2011) noted that good-quality, well-preserved silage should have a pH value of less than 4.2 to provide stability for the silage. During ensilage, fermentable carbohydrates in the form of water-soluble carbohydrates (WSC) are necessary as a carbon source for microorganisms. Thus, the addition of other feed ingredients to soybean curd residue provides not only the benefit of reducing moisture content but also the WSC to promote lactic bacteria fermentation.

Concerning the suitable ensiling period, Wang and Nishino (2008) observed the fermentative characteristics of soybean curd residue silage produced by two different ensiling periods: a short period (14 days) and a long period (56 days). They reported that prolonged ensiling (56 days) reduced the lactic acid content of the silage and increased the pH and acetate content. These observations suggest that in the long ensiling period, *Lactobacillus plantarum* or *Lactobacillus buchneri* metabolism was activated because these species are known to produce acetate from lactic acid under anaerobic conditions (Lindgreen et al., 1990). Although Wang and Nishino (2008) did not conduct animal feeding experiments with those different silages, it is widely understood that aerobically metabolized material is often toxic and should not be offered to animals. In another study, Amaha et al. (1996) developed a technique to preserve soybean curd residue silage by fermentation over 15 days. They obtained good-quality silage, as indicated by a low pH and high lactic acid content. Thus, it

seems that a short ensiling period (14 to 15 days) is the optimal fermentation period for soybean curd residue. Moreover, after the silo was left open for four days, the lactic acid content decreased and the pH increased (Amaha et al., 1996). Therefore, re-sealing after opening the silo is necessary to prevent the development of aerobic conditions and preserve the silage.

Soybeans naturally contain anti-nutritional properties (e.g., trypsin inhibitors). O'Toole et al. (1999) reported that these anti-nutritional properties are still present at low levels in soybean curd residue. However, Kondo et al. (2006) and Cao et al. (2009) suggested that the anti-nutritional properties of the feed material could be eliminated by fermentation. Indeed, a recent study by Rashad et al. (2011) revealed that fermentation could reduce the anti-nutritional properties of soybean curd residue. Moreover, they reported that fermented soybean curd residue has antioxidant activity similar to that of vitamin E, which could reduce the level of free radicals in the body and thus be useful as a healthy animal feed supplement.

Figure 1.2. Soybean curd residue silage manufacture (Hirakawa Food Co. Ltd., Iwate Prefecture, Japan)

Ruminant digestive system and metabolism

Ruminants have a unique digestive system with a capacious set of stomachs that harbor microorganisms capable to digest fibrous materials, such as cellulose. This allows ruminants to eat and digest plants, crops by-product and industrial residue, which may have a high fiber content and low nutritional value for simple-stomached animals. In this introductory chapter, the special features of the ruminant and the potential for quantitative description of ruminant physiology are described briefly. Anatomy of

ruminant digestive system includes the mouth, tongue, salivary glands (producing saliva for buffering ruminal pH), esophagus, stomach consisting four compartments (rumen, reticulum, omasum and abomasum), pancreas, gall bladder, small intestine (duodenum, jejunum and ileum) and large intestine (cecum, colon and rectum).

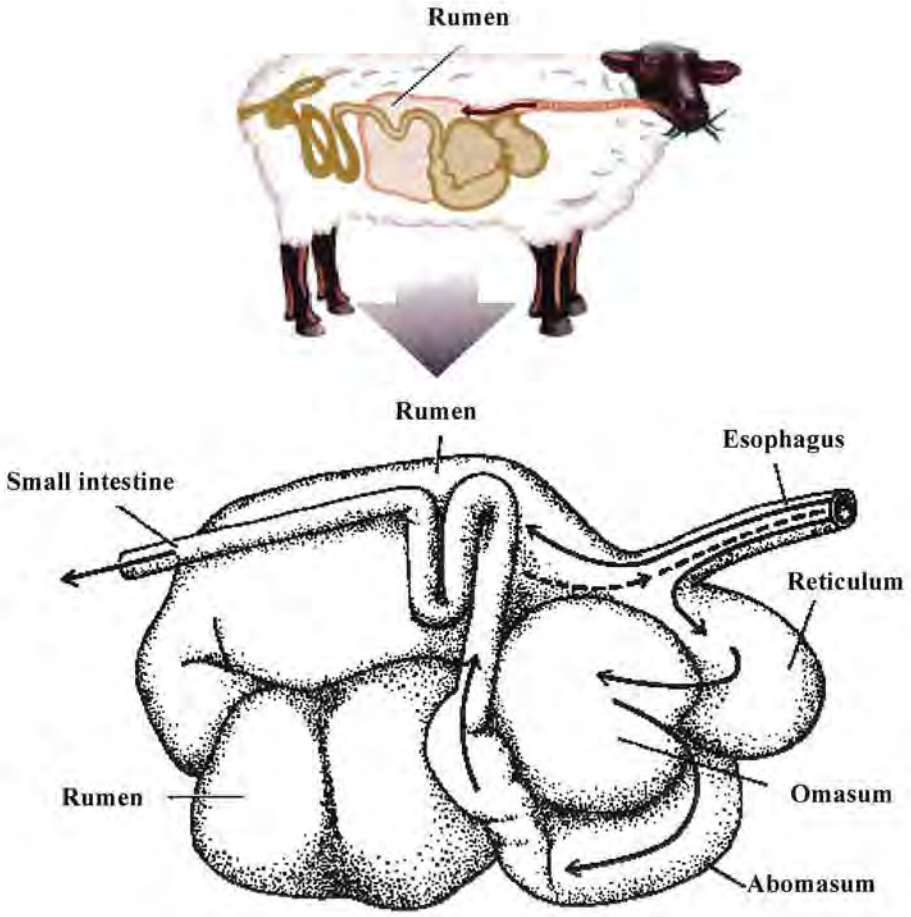


Figure 1.3. Ruminant digestive system. Diaphragmatic representation of four compartments of stomach (rumen, reticulum, omasum and abomasum) and the flow of digesta. Adapted from Church (1979).

In adult ruminant, the stomach occupies almost 75% of the abdominal cavity (McDonald et al., 2011). Feed, after being chewed during eating, enters the reticulo-rumen where it is subjected to microbial attack and to the mixing and propulsive forces

by the musculature contractions. This muscular activity results in the pattern of movement of digesta that is shown in Figure 1.3. The digesta passed from the reticulum to the omasum via a sphincter. From the omasum, digesta passed to the abomasum, the compartment that is almost similar with the stomach of non-ruminant. Acid and enzymes are secreted in the abomasum and are mixed with the digesta by the muscular activity of the organ. The small and large intestines follow the abomasum as further sites of nutrient absorption (Church, 1979; Djikstra et al., 2005; McDonald et al., 2011). Dietary carbohydrates, i.e. cellulose, hemicellulose, pectin, starch and soluble sugars are fermented by microbes in the rumen and yields short chain fatty acids, known as volatile fatty acids (VFA), microbial cells and gases such as methane and carbon dioxide (McDonald et al., 2011). In addition to dietary carbohydrate, dietary lipid and protein also can increase the concentration of VFA in the rumen (Church, 1979). The contribution from lipid is small because lipid normally presents in a small proportion of the diet, whereas protein can be a significant source of VFA, particularly if the diet containing high amount of ruminally degradable protein (RDP) (Djikstra et al., 2005). Acetate, propionate and butyrate are the predominant VFA, in which the concentration and relative proportion of the individual acid are related to the level of intake (Robinson, 1986) and the composition of the diet (Djikstra et al., 2005). In ruminants, VFA constitute the major source of energy and may provide up to 80% of the daily metabolizable energy absorption (Bergman, 1990). Both of total VFA concentration and the type of VFA formed can significantly affect the utilization of absorbed nutrient in ruminant, thus can affect the total milk production and milk composition in dairy cows (Sutton, 1989; Sarwar et al., 1992) and the growth rate in fattening ruminant (Ørskov et al., 1979).

Unlike the non-ruminants, ruminants have ability to synthesize protein in the rumen (microbial protein). Ruminal microbes are capable of utilizing non-protein nitrogen (NPN), primarily ammonia (NH_3) to synthesize microbial protein. However, some of dietary protein will escape ruminal degradation (ruminally undegradable protein, RUP). Some of the RUP will be digested in the small intestine and some of it will be excreted in feces. The end products from the digestion of dietary RDP in the rumen are NH_3 and microbial protein, whereas the end product from the digestion of RUP and microbial protein in the small intestine is AA (Church, 1979, Djikstra et al., 2005; McDonald et al., 2011).

Recent approaches in ruminant nutrition and physiology

As qualitative knowledge increased, it becomes possible to develop quantitative approaches to increase understanding on ruminant nutrition and physiology. Tracer method with radioactive or stable isotope is widely used in the metabolic study, both for human and animals. Compared to the radioactive isotope, the most obvious advantage of stable isotope is the little or no risk to human subject. To measure metabolic flux using stable isotope, the compound of interest must first be identified. The un-labeled nutrient (naturally occurring compound) is called 'tracee'. The labeled compound that is delivered to the subject experimentally is the 'tracer' (Wolfe and Chinkes, 2005; Waterlow., 2006). Typically, the tracer is chemically and functionally identical to the tracee. By following the fate of a tracer in the body, information can be obtained regarding the metabolism of the tracee. For example, the estimation of VFA production rates in ruminant can be determined by infusion of [1-^{13}C]acetate (Júnior et al., 2006) or [1-^{13}C]propionate (Martin et al., 2001), whereas the whole-body protein turnover can be assessed by infusion of [1-^{13}C]leucine (Leu) (Wolfe and Chinkes, 2005; Waterlow,

2006). Wolfe and Chinkes (2005) revealed that at some time (dependent on the kinetics) the tracer will be lost at the same time it appears and there will be no further change in the relative concentration (Figure 1.4). This situation is called an isotopic equilibrium or steady state condition, because a plateau in enrichment of the body pool of tracer is achieved.

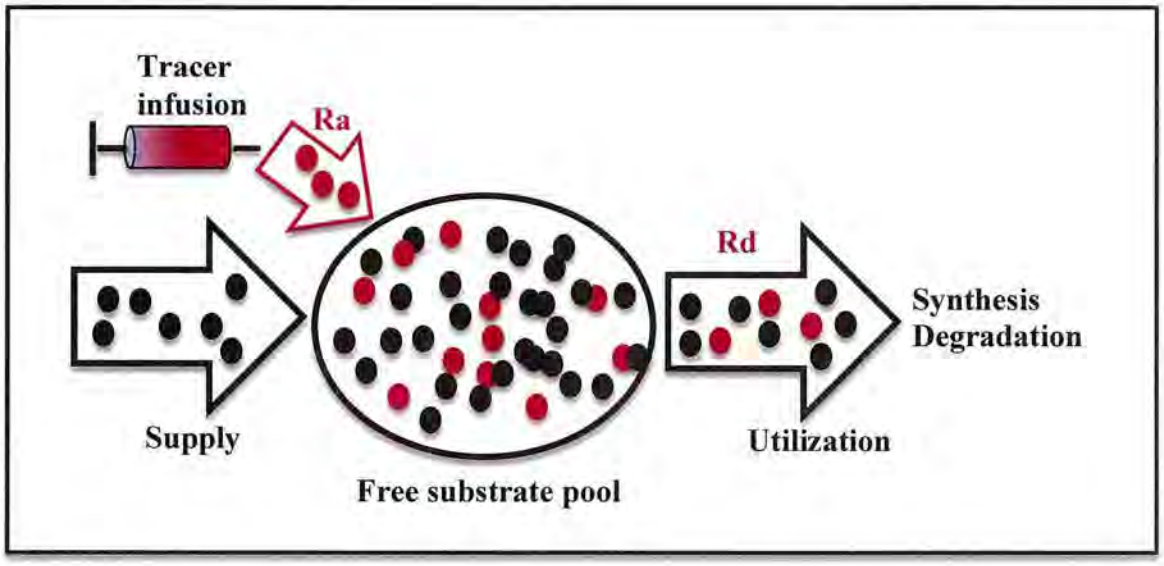


Figure 1.4. Schematic representation of a single pool, with Ra coming from constant infusion of tracer and the steady state is assumed as Ra = Rd.
(●) nutrient (tracee); (●) nutrient with isotopic tracer; Ra, rate of appearance; Rd, rate of disappearance.

In present study, sheep was used as a model to investigate the feeding effects of industrial food residue on the nutrient metabolism in the body. There are some advantages of using sheep as a model for the nutritional and physiological study. Sheep is large enough to support long period of frequent serial sampling of peripheral blood. Importantly, this can be done with individual cage and the sheep is freely standing and lying, thus avoid stress due to restraint. Moreover, sheep model has an intrinsic economic relevance for metabolic study (particularly for isotope dilution technique) and the findings from sheep can be transported to other livestock animals.

Objectives of the study

Driven by the fact that: i) soybean curd residue is a potentially useful ruminant feed given its relatively low price and abundant availability within Asian countries; ii) soybean curd residue silage can be stored for longer periods than raw soybean curd and has a high nutritive value because of its high soluble protein and fermentable carbohydrate contents; and iii) there is currently no data pertaining the nutritive value and beneficial effects of soybean curd residue silage consumption on ruminal fermentation and whole body nutrient metabolism, it was imperative to study the use of soybean curd residue silage.

Therefore, the aim of this thesis was to investigate the effects of soybean curd residue silage consumption on whole body nutrient metabolism in the ruminant. The following objectives were established and experimentally tested via a series of three different experiments (described in Chapter 2-4):

1. To determine the effects of inclusion of soybean curd residue silage in the diets on the ruminal characteristics, nitrogen balance, blood metabolites and plasma amino acid and glucose kinetics.
2. To compare the effects of feeding soybean curd residue silage and commercial concentrate on the ruminal characteristics, nitrogen balance, blood metabolites and plasma amino acid and glucose kinetics in sheep.
3. To investigate the effects of dietary level of soybean curd residue silage on plasma amino acid, acetate and glucose kinetics in sheep.

Chapter 2

The Effects of Inclusion of Soybean Curd Residue Silage in the Diets on Plasma Leucine and Glucose Kinetics, Blood Metabolites and Ruminal Characteristics in Sheep

Introduction

To optimize the amount of absorbable AA for ruminant productivity, one objective of the diet formulation is to provide adequate amounts of protein. Soybean curd residue silage has high levels of degradable protein and fermentable carbohydrates that may meet the nutritional needs of animals. Xu et al. (2001) reported no detrimental effect on ruminal fermentation when soybean curd residue silage was fed to sheep. Moreover, Cao et al. (2009) reported increased N digestibility in sheep fed whole-crop rice silage supplemented with soybean curd residue. Ruminants generally absorb only small amounts of glucose from the alimentary tract and rely on hepatic gluconeogenesis for their glucose supply. The major precursor of glucose in fed animals is propionate, which is produced by microbial fermentation in the rumen (Bergman, 1990). Some studies reported an increase in the ruminal propionate concentration in sheep fed soybean curd residue silage (Xu et al., 2001; Kakihara et al., 2004; Cao et al., 2009).

Based on these previous studies, it was hypothesized that feeding soybean curd residue silage could increase plasma AA and glucose kinetics due to its high N and fermentable carbohydrate contents. Therefore, first experiment was conducted to investigate the effects of inclusion of soybean curd residue silage in roughage diet on the ruminal fermentation characteristics, N balance and the plasma kinetics of Leu and glucose in sheep using the isotope dilution technique of [1-^{13}C]Leu and [U-^{13}C]glucose.

Materials and Methods

Animals, dietary treatments and feeding

The handling of the experimental animals, including blood sampling, was conducted according to the guidelines established by the Animal Care Committee of Iwate University. Six crossbred (Suffolk × Corriedale) sheep, all approximately 4 years of age and initially of 50 ± 5 kg of body weight (BW) were used. Two different dietary treatments were tested, one was mixed orchardgrass and reed canarygrass hay (Hay diet) and another one (SCRS diet) was mixed hay plus soybean curd residue silage containing 15% beet pulp at a ratio of 8:2 on a DM basis. The soybean curd residue silage was purchased from Hirakawa Food Co. Ltd. (Japan), placed into 90 L polyvinyl silo, compacted, and tightly sealed to ensure anaerobic conditions over 15 days ensiling period. After 15 days, the silo was opened and the silage was fed to sheep. The silo was then re-sealed and stored at 4°C until the next feeding. The chemical compositions of dietary treatments are given in Table 2.1. The diets were formulated slightly above the maintenance level (NRC, 1985; NARO, 2009), as shown in Table 2.2. The sheep were fed twice per day at 10.00 and 22.00 h and had *ad libitum* access to water.

The experiment followed a crossover design with a period of 21 days. The layout of experiment is illustrated in Figure 2.1. The experimental period consisted of 14 days of adaptation to the diets and 7 days of sample collection. The sheep were housed in individual pens during the adaptation period and then moved to individual metabolic cages in a controlled-environment room at an air temperature of 23°C and 70% relative humidity, with light provided from 09.00 to 23.00 h. The sheep were weighed at starting of the experiment, on day 8, 15 and after the finishing of each dietary treatment.

Table 2.1. Chemical compositions of the dietary treatments

	Hay diet (Mixed hay)[1]	SCRS diet[2]	Soybean curd residue silage[3]
DM (g/kg)	894	781	327
CP (g/kg DM)	110	132	220
EE (g/kg DM)	34	49	110
NDF (g/kg DM)	680	570	134
NFC (g/kg DM)	76	152	456
Ash (g/kg DM)	100	96	80
ME[4] (Mcal/kg DM)	1.97	-	3.32

[1] Mixed hay of orchardgrass and reed canarygrass.
[2] SCRS diet consisted of 80% mixed hay plus 20% soybean curd residue silage on DM basis.
[3] Soybean curd residue silage containing 15% beet pulp (Hirakawa Food Co., Ltd., Iwate, Japan).
[4] Metabolizable energy of mixed hay was calculated as the proportion of orchardgrass and reed canarygrass (60:40) according to NRC (1985). Metabolizable of soybean curd residue silage was calculated as the proportion of soybean curd residue and beet pulp (85:15) in the silage according to NARO (2009).

Table 2.2. Diet formulation and intakes of CP and ME of the dietary treatments

	Treatment[1]	
	Hay diet	SCRS diet
Mixed hay (g/kg $BW^{0.75}$/day)	67.0	48.5
Soybean curd residue silage (g/kg $BW^{0.75}$/day)	0	12.1
CP intake (g/kg $BW^{0.75}$/day)	7.4	8.0
ME intake[2] (Mcal/kg $BW^{0.75}$/day)	132	135

[1] Treatment: Hay diet, mixed hay (orchardgrass and reed cannarygrass); SCRS diet, mixed hay supplemented with soybean curd residue silage (SCRS diet) at a ratio of 80:20 on DM basis.
[2] Assumed from NRC (1985).

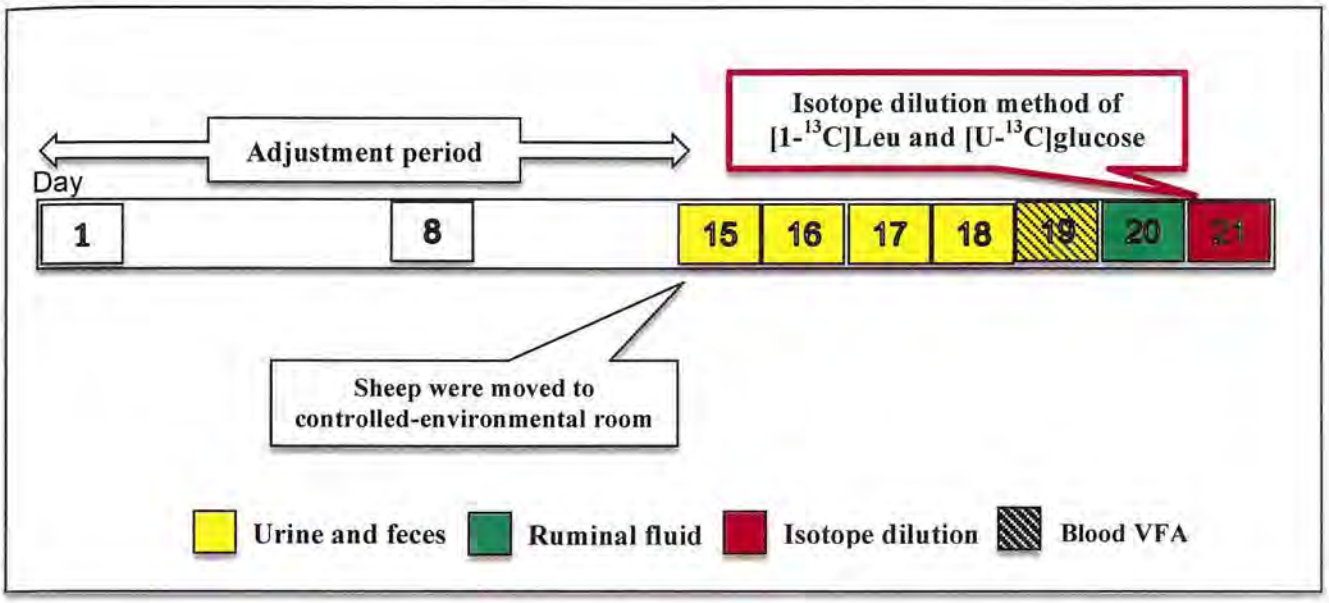


Figure 2.1. The experimental layout showing sampling protocol.

Urine and feces

Urine and feces were collected separately for 5 successive days (from day 15 to 19) at 24-h intervals using a 3-mm plastic screen as a separator. Urine was collected in a bucket containing 50 mL of 6 N H_2SO_4 to prevent the loss of N. The urine volume was recorded, and a subsample (50 mL) was stored at -30°C prior to further analysis. The feces were dried in a forced-air oven (60°C for 48 h), ground through a 1-mm mesh and stored at room temperature prior to further analysis.

Blood VFA

On day 19 of each trial, a polyvinyl catheter was inserted in the right jugular vein. The blood samples (2 mL) were drawn hourly from before feeding (0 h) to 6 h after feeding to determine the blood VFA concentrations. The blood samples were transferred into heparinized tubes and temporarily stored on ice before deproteinization. The blood samples were deproteinized by adding 2 mL of 100 g/L sodium tungstate

solution and 2 mL of 0.34 M H_2SO_4, and then kept at room temperature overnight (Fujita et al., 2006). The supernatant fluid obtained after centrifugation at 3,000 × *g* for 10 min at 4°C (RS-18V, Tomy, Japan) was stored at -30°C until further analysis.

Ruminal fluid

Ruminal fluid (50 mL) was collected before feeding (0 h) and at 3 and 6 h after feeding via a stomach tube inserted orally on day 20. Approximately 200 mL of ruminal fluid was collected at each time. The pH value of the ruminal fluid was measured immediately after collection with a pH meter (HM-10P, Toa Electronics Ltd., Japan). The liquid fraction was separated by centrifugation at 8,000 × *g* for 10 min at 4°C. An aliquot (5 mL) of ruminal fluid was used to measure the ruminal VFA concentration, and another 1 mL was acidified with 1 mL of 0.1 mol/L HCl to stop the microbial activity and prevent the loss on NH_3 for ruminal NH_3 concentration measurement. All samples were kept at -30°C prior to further analysis.

Isotope dilution procedures

A primed-continuous infusion of [1-^{13}C]Leu and [U-^{13}C]glucose was conducted on day 21 of each treatment to assess plasma Leu and glucose kinetics in the sheep. Polyvinyl catheters were inserted in both jugular veins of each sheep on the morning of the day on which the isotope dilution procedure was conducted. A saline solution containing 7.2 μmol/kg $BW^{0.75}$ of [1-^{13}C]Leu (L-leucine-1-^{13}C, 99 atom% excess ^{13}C; Cambridge Isotope Laboratories, USA) and 2.9 μmol/kg $BW^{0.75}$ of [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. The tracer solution was

continuously infused over 4 h period (between 3 to 7 h after feeding) immediately after the priming injection using a multichannel peristaltic pump (AC-2120, Atto, Japan) at a rate of 7.2 μmol/kg $BW^{0.75}$/h and 2.9 μmol/kg $BW^{0.75}$/h for [1-^{13}C]Leu and [U-^{13}C]glucose, respectively, through the same catheter. The infusion rate of the tracer solution was recorded every 30 min throughout the infusion period.

The blood samples (6 mL) were taken from the left jugular vein immediately before the priming injection and at 30-min intervals during the last 2 h of the isotope infusion period (5 to 7 h after feeding). Blood samples were placed in heparinized tubes and temporarily stored on ice. The plasma from the whole blood samples was separated by centrifugation at 8,000 × *g* for 10 min at 4°C and was stored at -30°C prior to further analyses.

Chemical analysis

The chemical compositions of the diets were analyzed using the methods described by the Association of Official Analytical Chemists (AOAC, 1990). The neutral detergent fiber (NDF) contents in soybean curd residue silage and mixed hay were determined according to Van Soest et al. (1991) using the Foss Analytical FiberCap system (FiberCap^{TM} 2021/2023, Foss, Sweden). Briefly, samples were weighed (1 g) in the capsules and extracted by boiling the capsules in the beaker containing NDF solution for 1 h. Then, the capsules were washed in a hot water (3 × 0.5 min), rinsed in methanol (2 × 0.5 min) and dried in the air oven (130°C for 3 h). After dried, the capsules were weighed and then ignited in the muffle furnace (600°C for 4 h). The compositions of 1 L of NDF solution were listed in Table 2.3.

Table 2.3. The ingredient of the NDF solution

Item	Amount (in 1 L of solution)
Disodium ethylenediaminetetraacetate (EDTA)	18.61 g
Sodium borate decahydrate	6.81 g
Sodium lauryl sulphate	30 g
Disodium hydrogenphosphate	4.56 g
Triethylene glycol	10 mL

The fermentation products of soybean curd residue silage were determined from cold-water extracts (Cao et al., 2009). The silage pH was measured using the pH meter. The VBN content in the silage was determined based on the steam distillation method according to Dhaouadi et al. (2007). Briefly, 10 mL of soybean curd residue silage extract was added to 3.5 mL of 20% NaOH solution and a few drops of phenolphthalein indicator. The distillate was collected in a conical flask containing 20 mL of 4% boric acid and a few drops of Tashiro indicator and subsequently titrated against 0.01 N HCl. The lactic acid concentration in the silage was determined using the colorimetric method according to Taylor (1996). The silage extract (300 µL) was mixed with 200 µL of distillated water and 3 mL of concentrated H_2SO_4, followed by incubation at 95-100°C for 10 min prior to the addition of 50 µL $CuSO_4$ and 100 µL of phenolphthalein reagent. The absorbance was measured at 570 nm using a spectrophotometer (U-1000, Hitachi, Japan). To assess the silage quality, the V-score and Flieg point were calculated from the fermentative components as described by Takahashi et al. (2005) and Yilmaz et al. (2009). The VFA concentrations in the soybean curd residue silage, ruminal fluid and deproteinized-blood samples were determined after steam distillation.

Each sample (silage extract, ruminal fluid or deproteinized-blood) was mixed with 20% H_2SO_4 and $MgSO_4$, followed by steam distillation. The distilled samples were titrated against 0.1 N NaOH for the ruminal fluid sample and 0.01 N NaOH for the silage extract and blood samples. The distillates were dried in the air oven at 70°C. The VFA were measured by gas chromatography (HP-5890, Hewlett Packard, USA), and the molar concentration of each VFAs were calculated from the ratio of standard area and sample area, using crotonic acid as an internal standard. The ruminal NH_3 concentration was determined as described by Weatherburn (1967) by mixing the ruminal fluid sample with phenol and hypochlorite reagents and then incubating the sample in a water bath at 37°C for 20 min. The absorbance was measured by a spectrophotometer (V-630 BIO, JASCO, Japan) at 625 nm. The N contents in the diet, urine and feces were analyzed using the Kjeldahl method with the Foss Kjeltec System (Kjeltec 2100, Foss, Sweden). The sample (diet, urine or feces) was digested by concentrated H_2SO_4 in the presence of a catalyst that promotes the conversion of NH_3 to NH_4^+. The NH_4^+ molecules were then converted into NH_3 gas, heated and distilled. The NH_3 gas was led into a trapping solution. Finally, the amount of trapped NH_3 was determined by titration with a standard solution and calculated automatically by the Foss Kjeltec system.

The concentrations of plasma Leu and α-ketoisocaproic acid (α-KIC) and the plasma enrichments of [1-^{13}C]Leu and α-[1-^{13}C]KIC were determined according to the procedure of Rocchiccioli et al. (1981) and Calder and Smith (1988). Briefly, 1 mL of plasma was deproteinized by adding 1 mL of 4% sulfosalicylic acid (SSA) and the internal standard, 100 μL of n-Leu (0.5 mmol/L) and 100 μL of ketovaleric acid (KVA) (0.05 mmol/L). After centrifugation at 12,000 × *g* for 10 min at 0°C, the supernatant was applied to a column containing 0.5 mL of cation exchange resin (Dowex 50W × 8,

hydrogen form), and the column was washed with distilled water (2 × 0.5 mL). The eluent obtained was submitted to α-KIC analysis. Subsequently, 4 N NH_4OH was passed through the column (2 × 1 mL) and washed again with distilled water (1 × 1 mL). A 0.5 mL aliquot of the resulting eluent was collected in a screw-capped glass tube and dried in a dessicator containing H_2SO_4 for Leu analysis. From the α-KIC fraction, 1 mL of eluent was mixed with 0.5 mL of 1% o-phenylenediamine in 4 M HCl solution in a screw-capped glass tube and heated for 1 h at 90°C followed by cooling for 1 h at room temperature. Then, 2 mL of ethyl acetate was mixed by vigorous shaking for 1 min and centrifuged at 1,000 × *g* for 10 min at 4°C. The supernatant was separated and dried using anhydrous Na_2SO_4 for 2 h, and the supernatant was then placed in a new screw-capped glass tube and dried with N gas. After all of the samples (for Leu and α-KIC analysis) were dried, 25 μL of acetonitrile and 25 μL of *N*-methyl-*N*-t-butyl-dimethylsilyltrifluoroacetamide (MTBSTFA) were added to each screw-capped glass tube. The tubes were capped and then heated at 80°C for 20 min in the air oven. The concentrations of plasma Leu and α-KIC and the enrichments (atom % excess) of plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC were determined by gas chromatography-mass spectrometry (GC-MS, QP-2010, Shimadzu, Japan) with selected ion monitoring. The following ions were monitored: m/z 302 and 303 for Leu, m/z 302 for n-Leu, m/z 245 for KVA, m/z 259 and 260 for α-KIC.

The plasma [U-^{13}C]glucose enrichment (atom % excess) was measured as described by Tserng and Kalhan (1983), with the slight modification described by Fujita et al. (2006). The plasma (1 mL) was deproteinized by adding 1 mL of 4% SSA. After centrifugation at 12,000 × *g* for 10 min at 0°C, the supernatant was purified through a tandem column containing 0.5 mL cation exchange resin (Dowex 50W × 8, hydrogen

form) and 1 mL anion exchange resin (Dowex 1 × 8, acetate form). After elution with distilled water (2 × 0.5 mL), the glucose fractions were pooled, dried and converted to pentacetate derivative with acetic anhydride and pyridine. The samples were heated in the air oven for 1 h at 90°C followed by cooling on ice for 3 min. Then, 2 mL of distillated water and 1 mL of chloroform were added and mixed by vigorous shaking for 1 min (repeated 3 times). The two phases were then separated by centrifugation at 3,000 × *g* for 5 min at 3°C. The upper layer was discarded and the sample was then placed in a new glass tube and dried with N gas. After all of the samples were dried, 100 μL of chloroform were added into each glass tube. The derivative was analyzed with GC-MS with ionization using a 20 eV electron beam, and the fragment was monitored at m/z 314 and m/z 319.

The plasma glucose concentration was determined using the glucose oxidase method described by Huggett and Nixon (1957). The plasma (200 μL) was deproteinized by adding 1.8 mL of trichloroacetic acid, and the liquid fraction was separated by centrifugation at 3,000 × *g* for 10 min at 4°C. The liquid obtained was combined with the mixed enzyme-oxygen acceptor reagents as follows: 200 mg/L glucose oxidase, 60 mg/L peroxidase and 10 mg of o-dianisidine in 10 mL of 95% ethanol. The sample was incubated at 37°C for 1 h in a water bath and then the absorbance was measured at 440 nm using the spectrophotometer. To determine the concentrations of plasma AA, NH_3 and urea, the plasma was deproteinized by mixing 1 mL of plasma with 1 mL of 3% SSA. The supernatant was separated by centrifugation at 3,000 × *g* for 10 min at 4°C and then passed via syringe through a 0.45 μm cellulose acetate filter (Advantec, Japan). Approximately 50 μL of this filtrate was used for the determination of plasma AA, NH_3 and urea concentrations 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). The plasma (50 μL) was mixed with 1 mL of color A solution containing acyl-CoA synthetase, coenzyme A (CoA), adenosine-5-triphosphate disodium salt (ATP), 4-aminoantipyrine and ascorbate oxidase, followed by incubation at 37°C for 10 min in a water bath. After incubation, 1 mL of color B solution containing acryl-CoA oxidase and peroxidase was added into each sample and then incubated again at 37°C for 10 min in a water bath and finally the absorbance was measured at 550 nm using the spectrophotometer.

Calculation and statistical analysis

Mean values with standard error of the mean (SEM) for all data are given. The turnover rates of plasma Leu and glucose (LeuTR and GluTR) were calculated according to Wolfe and Chinkes (2005).

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

where I is the infusion rates of [1-^{13}C]Leu or [U-^{13}C]glucose and E is the isotope enrichment of plasma α-[1-^{13}C]KIC, a metabolite of [1-^{13}C]Leu, or plasma [U-^{13}C]glucose during the steady state, respectively.

The whole body protein flux (WBPF) was calculated by dividing the LeuTR by 0.066 (Leu concentration in sheep carcass protein suggested by Harris et al. (1992)). Then, whole body protein synthesis (WBPS) and degradation (WBPD) were calculated from the relationship between the WBPF and N balance according to Schroeder et al. (2006).

WBPF = LeuTR / 0.066

WBPS = WBPF – (urinary N excretion x 6.25)

WBPD = WBPF – (N absorption x 6.25)

All data were analyzed using the MIXED procedure of SAS (1996). The fixed effects in the model included period, diet, and period x diet interaction, and the random effect was sheep. The result was considered significant at $P<0.05$, and the tendency was defined as $0.05 \leq P < 0.10$. Repeated statements and Tukey's adjustment were used for the time course of changes. The results were considered significant at $P<0.05$.

Results

Silage quality, body weight gain and nitrogen balance

The silage pH was 4.11, and organic acids (lactic acid, acetate, propionate and butyrate) were detected in the silage (Table 2.4). The feeding effects of soybean curd residue silage on the BW gain and N balance are shown in Table 2.5. The BW gain during experimental period tended to be higher ($P=0.06$) for sheep fed the SCRS diet than those fed the Hay diet. The sheep fed the SCRS diet exhibited a higher ($P<0.0001$) N intake than sheep fed the Hay diet. Fecal N excretion was lower ($P=0.001$) and urinary N excretion was higher ($P=0.008$) for sheep fed the SCRS diet than those fed the Hay diet. The N absorption and digestibility were higher ($P<0.0001$ and $P=0.001$) and N retention tended to be higher ($P=0.07$) for the SCRS diet than for the Hay diet.

Table 2.4. The fermentative characteristics in the soybean curd residue silage

Item	Amount
Moisture (g/kg)	673
pH	4.11
Lactic acid (g/kg FM)	15.4
Acetate (g/kg FM)	1.1
Propionate (g/kg FM)	0.03
Butyrate (g/kg FM)	0.003
VBN (g/kg total N)	60
Flieg point	100
V-score	98

Table 2.5 The effects of feeding soybean curd residue silage on BW gain and N balance in sheep[1]

Item	Treatment[2]		SEM[3]	*P value*
	Hay diet	SCRS diet		
BW gain (kg/day)	0.08	0.18	0.04	0.06
N intake (g/kg $BW^{0.75}$/day)	1.17	1.28	0.01	<0.0001
Fecal N (g/kg $BW^{0.75}$/day)	0.47	0.43	0.01	0.001
Urinary N (g/kg $BW^{0.75}$/day)	0.42	0.51	0.02	0.008
N absorption (g/kg $BW^{0.75}$/day)	0.70	0.85	0.01	<0.0001
N retention (g/kg $BW^{0.75}$/day)	0.29	0.33	0.02	0.07
N digestibility (%)	61.0	66.1	1.2	0.001

[1] Values represent the mean of 6 sheep.
[2] Hay diet, mixed hay of orchardgrass and reed canarygrass; SCRS diet, mixed hay plus soybean curd residue silage (at a ratio of 8:2).
[3] SEM, standard error of the mean.

The changes in the pH value and the concentrations of NH_3 and VFA in the rumen after feeding are illustrated in Figure 2.2. The mean value of ruminal pH and the mean concentrations of VFA and NH_3 across all sampling time are presented in Table 2.6. For both diets, the pH values decreased at 3 h after feeding and did not return to the initial values by 6 h after feeding. The dietary treatments did not affect the ruminal pH (P=0.60). The concentrations of NH_3, total ruminal VFA, acetate, propionate, butyrate, and valerate increased (P<0.05) at 3 h after feeding and subsequently decreased (P<0.05). Higher (P=0.01) ruminal NH_3 concentrations were found in sheep fed the SCRS diet than those fed the Hay diet. The concentrations of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate in the rumen were higher (P=0.03, P=0.01, P=0.02, P<0.001, P=0.01, and P=0.03, respectively) for the SCRS diet than the Hay diet. It is resulted in higher (P=0.03) total ruminal VFA concentration for the SCRS diet than the Hay diet. The molar ratio of acetate to propionate tended to be lower (P=0.09) for the SCRS diet than the Hay diet.

The postprandial changes in the concentrations of blood VFA are illustrated in Figure 2.3. The mean concentrations of blood VFA across all sampling time are presented in Table 2.7. The concentrations of total blood VFA, acetate and propionate were differed within the sampling time (P<0.001, P<0.001 and P=0.001, respectively), whereas butyrate concentration in the blood only tended (P=0.05) to be different within the sampling time. The blood acetate and propionate values measured for the two diets increased gradually from the values measured before feeding, reached a peak level at 3 h after feeding and then decreased gradually. The total blood VFA and acetate concentrations tended to be higher (P=0.05 and P=0.08) for sheep fed the SCRS diet

than those fed the Hay diet, whereas the blood propionate and butyrate concentrations did not differ (P=0.11 and P=0.16) between the diets.

The plasma AA, NH_3, urea and NEFA concentrations measured from the pre-infusion blood samples are shown in Table 2.8. The concentrations of plasma AAs did not differ (P>0.05) between the treatments, except that the plasma glutamic acid concentration was lower (P=0.01) for the SCRS diet than for the Hay diet. The plasma NH_3 and urea concentrations were higher (P=0.003 and P=0.02) for the SCRS diet than for the Hay diet, whereas the plasma NEFA concentrations did not differ (P=0.70) between the two diets.

The plasma Leu and α-KIC concentrations and plasma α-[1-^{13}C]KIC enrichment were virtually constant during the last 2 h of isotope infusion (Figure 2.4). The plasma Leu and α-KIC concentrations and the plasma LeuTR (Table 2.9) were not significantly influenced (P=0.57, P=0.40, P=0.29, respectively) by the dietary treatment. The WBPS and WBPD also did not significantly influenced (P=0.38 and P=0.34) by the treatments.

The plasma glucose concentration and [1-^{13}C]glucose enrichment were also constant during the last 2 h of isotope infusion (Figure 2.5). The plasma glucose concentration tended to be higher (P=0.06) in the SCRS diet than in the Hay diet and plasma GluTR was not significantly influenced (P=0.17) by the diets (Table 2.9).

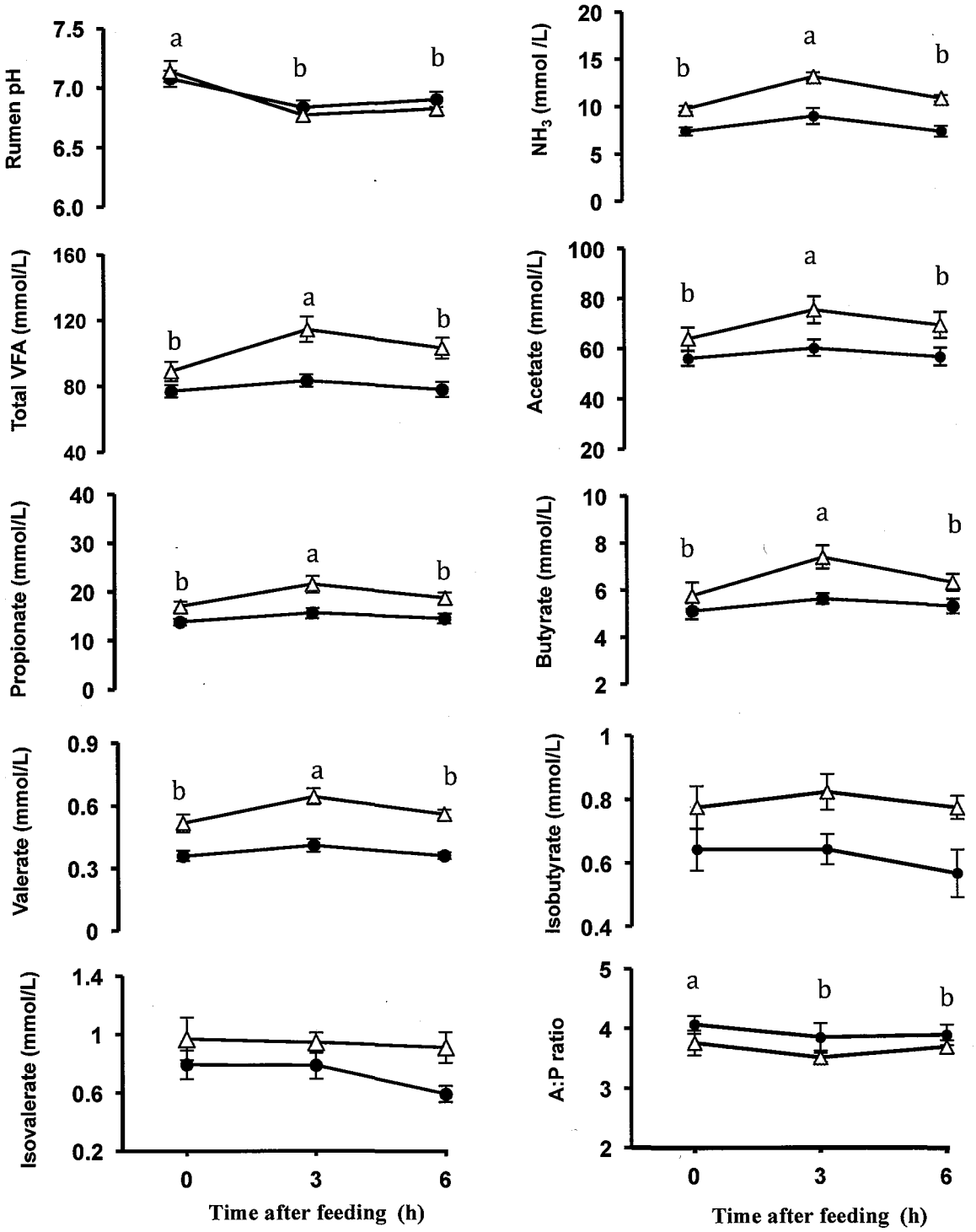


Figure 2.2. Time course changes of the ruminal characteristics in sheep fed the Hay diet (●) and the SCRS diet (Δ). The values are expressed as the mean ± SEM for n = 6. Different letters (a, b, c) indicate significant difference ($P<0.05$) between times after feeding.

Table 2.6. The effects of feeding soybean curd residue silage on ruminal pH, and the concentrations of ruminal NH_3 and VFA in sheep[1]

Item	Treatment[2]		SEM[3]	*P value*		
	Hay diet	SCRS diet		Treatment	Time	Treatment x Time
pH	6.94	6.83	0.04	0.60	0.001	0.48
NH_3 (mmol/L)	7.9	11.3	0.4	0.01	0.001	0.10
Total VFA (mmol/L)	79.5	97.6	2.2	0.03	0.002	0.68
Individual VFA concentrations (mmol/L)						
Acetate	58.0	69.6	1.1	0.03	0.04	0.27
Propionate	14.7	19.1	1.0	0.01	0.01	0.19
Butyrate	5.3	6.5	0.3	0.02	0.01	0.16
Valerate	0.4	0.6	0.02	<0.001	0.01	0.33
Isobutyrate	0.6	0.8	0.1	0.01	0.02	0.76
Isovalerate	0.7	0.9	0.1	0.03	0.18	0.45
Acetate:propionate	3.9	3.6	0.1	0.09	0.06	0.76

[1] Values represent the means from 3 sampling periods (0, 3, and 6 h after feeding) for 6 sheep.
[2] Hay diet, mixed hay of orchardgrass and reed canarygrass; SCRS diet, mixed hay plus soybean curd residue silage (at a ratio of 8:2).
[3] SEM, standard error of the mean.

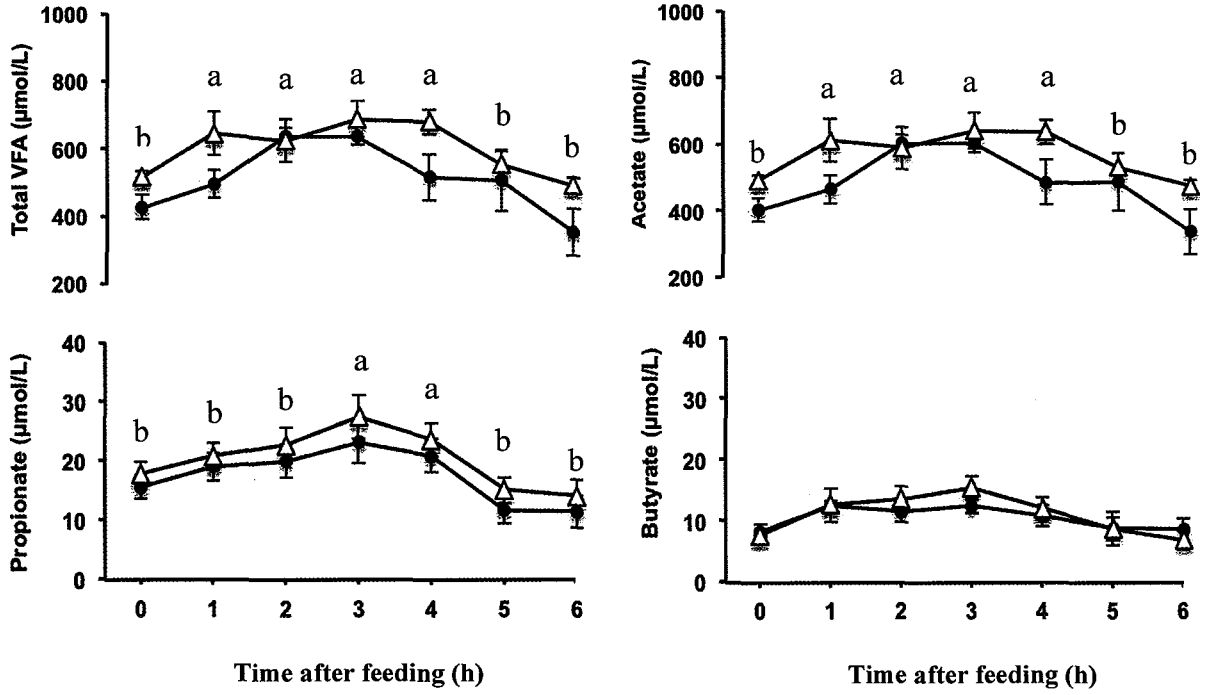


Figure 2.3. Postprandial changes in the blood VFA concentrations in sheep fed the Hay diet (●) and those fed the SCRS diet (Δ). The values are expressed as the mean ± SEM for n = 6. Different letters (a, b) indicate significant differences (P<0.05) between the times after feeding.

Table 2.7. The effects of feeding soybean curd residue silage on blood VFA concentration in sheep[1]

Item	Treatment[2]		SEM[3]	*P value*		
	Hay diet	SCRS diet		Treatment	Time	Treatment x Time
Total VFA (μmol/L)	510	600	30	0.05	<0.001	0.15
Acetate (μmol/L)	483	569	20	0.06	<0.001	0.13
Propionate (μmol/L)	17	20	2	0.11	0.001	0.20
Butyrate (μmol/L)	10	11	2	0.56	0.05	0.81

[1] Values represent the means from 7 sampling periods (0, 1, 2, 3, 4, 5, and 6 h after feeding) for 6 sheep.
[2] Hay diet, mixed hay of orchardgrass and reed canarygrass; SCRS diet, mixed hay plus soybean curd residue silage (at a ratio of 8:2).
[3] SEM, standard error of the mean.

Table 2.8. The effects of feeding soybean curd residue silage on plasma AA, NH_3, urea and NEFA concentrations at pre-infusion of isotope in sheep[1]

Item	Treatment[2]		SEM[3]	*P* value
	Hay diet	SCRS diet		
Essential AA (μmol/L)				
Threonine	264	261	18	0.91
Valine	298	270	12	0.15
Methionine	28	20	2	0.14
Isoleucine	106	92	6	0.20
Leucine	146	124	9	0.17
Phenylalanine	66	60	4	0.35
Histidine	62	58	2	0.32
Lysine	151	118	13	0.19
Non-essential AA (μmol/L)				
Serine	176	144	14	0.22
Asparagine	116	104	10	0.40
Glutamic acid	96	74	5	0.01
Glutamine	310	280	12	0.23
Glycine	606	535	35	0.31
Alanine	198	190	11	0.64
Tyrosine	96	91	7	0.53
Arginine	194	156	12	0.11
Proline	112	102	10	0.47
NH_3 (μmol/L)	142	212	6	0.003
Urea (mmol/ L)	4.40	6.83	0.2	0.02
NEFA (μEq/ L)	124	112	9	0.70

[1] Values represent the means for 6 sheep.
[2] Hay diet, mixed hay of orchardgrass and reed canarygrass; SCRS diet, mixed hay plus soybean curd residue silage (at a ratio of 8:2).
[3] SEM, standard error of the mean.

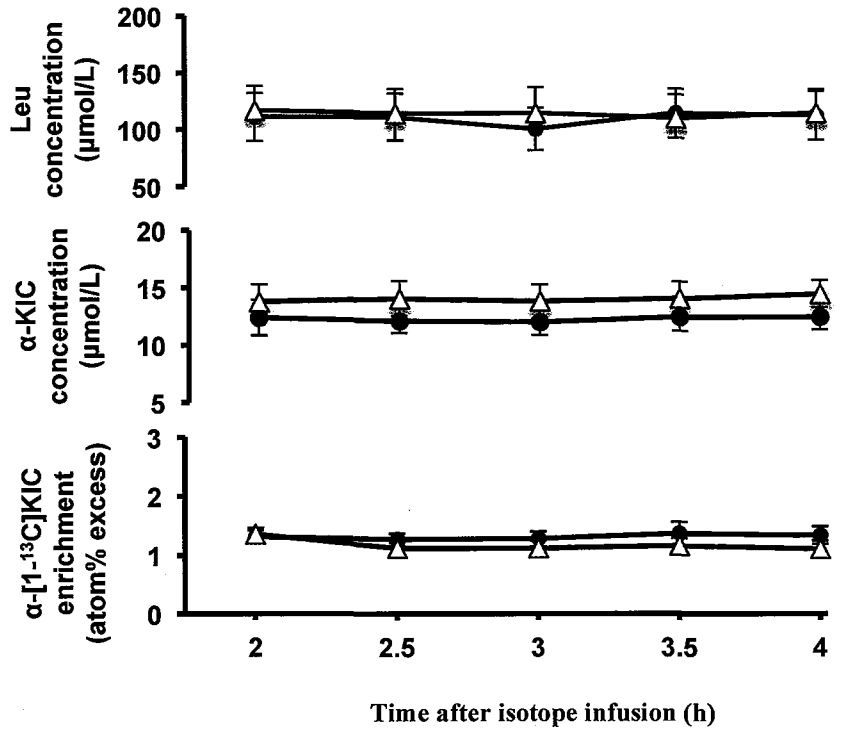


Figure 2.4. Time course changes of plasma Leu and α-KIC concentrations and α-[1-^{13}C]KIC enrichment during the last 2 h of isotope infusion in sheep fed the Hay diet (●) and those fed the SCRS diet (Δ).

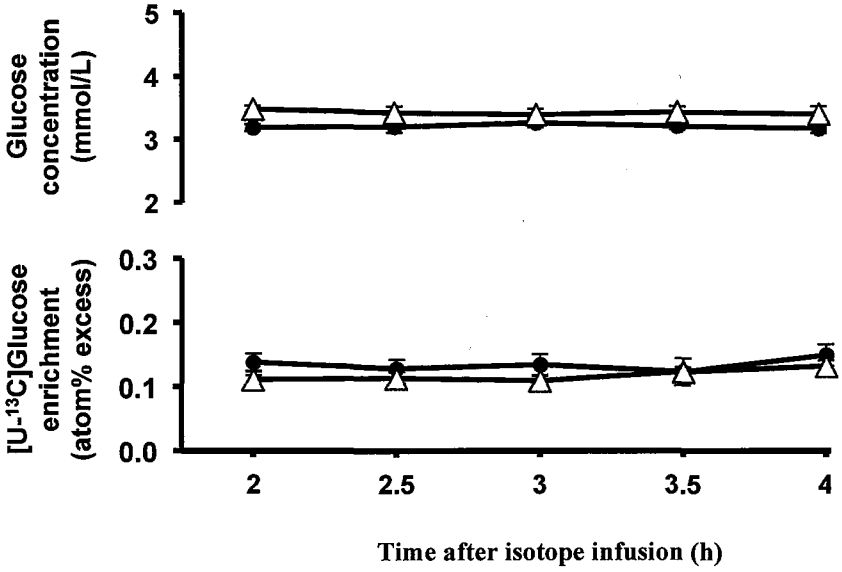


Figure 2.5. Time course changes of plasma glucose concentration and [U-^{13}C]glucose enrichment during the last 2 h of isotope infusion in sheep fed the Hay diet (●) and those fed the SCRS diet (Δ).

Table 2.9. The effects of the source of N supplementation on plasma Leu and glucose kinetics in sheep[1]

Item	Treatment[2]		SEM[3]	*P* value
	Hay diet	SCRS diet		
Leu kinetics				
Leu concentration (μmol/L)	112	116	4	0.57
α-KIC concentration (μmol/L)	12.9	14.3	1.4	0.40
LeuTR (μmol/kg $BW^{0.75}$/h)	446	514	36	0.29
WBPS (g/kg $BW^{0.75}$/day)	18.6	21.3	2.2	0.38
WBPD (g/kg $BW^{0.75}$/day)	16.8	19.7	2.1	0.34
Glucose kinetics				
Glucose concentration (mmol/L)	3.13	3.37	0.10	0.06
GluTR (mmol/kg $BW^{0.75}$/h)	1.76	2.10	0.20	0.17

[1] Values represent the means for 6 sheep.
[2] Hay diet, mixed hay of orchardgrass and reed canarygrass; SCRS diet, mixed hay plus soybean curd residue silage (at a ratio of 8:2).
[3] SEM, standard error of the mean.

Discussion

Silage quality

Beet pulp was added to soybean curd residue silage (at a ratio soybean curd residue:beet pulp = 85:15) to adjust the moisture content. The DM content in our silage (32.7%) was comparable to that of soybean curd residue silage containing peanut hull (34.5%, at a 78:22 ratio) and higher than soybean curd residue silage without any additive (18.5%), as reported by Yang et al. (2005). Soybean curd residue silage used in the current study was well preserved, as indicated by its low pH, high lactic acid

content, high V-score and Flieg point. The fermentation characteristics of the soybean curd residue silage analyzed in this study were comparable with those observed previously (Xu et al., 2001). Soybean curd residue silage is naturally rich in soluble carbohydrates. Thus, it provides sufficient sugar for the production of organic acids, primarily lactic acid, by microbial fermentation. Only a small quantity of butyric acid was found in the silage. This compound is considered to represent a secondary product of anaerobic fermentation, as suggested by Wang and Nishino (2008). However, the presence of butyric acid did not influence the overall quality of the silage. The efficiency of fermentation can also be judged by the VBN content in the silage because the VBN content reflects the degree of protein degradation. The VBN content in our silage (68 g/kg total N) was in the normal range (well-preserved silage should contain less than 100 g VBN/kg total N), as described by McDonald et al. (2011).

Nitrogen utilization

It is widely hypothesized that N utilization from food by-products is likely affected by the quality of the materials and processing methods (Yang et al., 2005; Cao et al., 2009). Fermentation process increases the solubility of N fractions in the soybean curd residue silage through the extensive hydrolysis of N compound and this process may affect the N metabolism in the animal. Low fecal N excretion in the SCRS fed sheep indicated that soybean curd residue silage might contain large amounts of degradable N. Previously, Swanson et al. (2000) and Castillo et al. (2001) reported a decrease in fecal N excretion in sheep and dairy cows with increasing dietary RDP intakes. Moreover, although higher urinary N excretion was found in sheep fed the SCRS diet than the Hay diet, the SCRS-fed sheep tended to retain more N, due to their

higher N digestibility. Similar responses to soybean supplementation have been observed in goats and sheep (Kadzere and Jingura, 1993; Cao et al., 2009; Foster et al., 2009). Results indicating that soybean curd residue silage have a positive effect on N utilization in sheep. Hence, this by-product should not be considered as waste but as potential protein source for sheep.

Ruminal fermentation characteristics

Although soybean curd residue silage contained high levels of readily fermentable carbohydrate compounds, which often resulted in a rapid decrease in the ruminal pH (McDonald et al., 2011), the present study demonstrated that the inclusion of soybean curd residue silage in the diet did not adversely affect ruminal pH. Previous studies revealed that diet rich in fermentable carbohydrate (Bergman, 1990) and protein (Djikstra, 1994; Cunningham et al., 1996) increased the concentration of VFA in the rumen. The current finding was agreed with them, because concentrations of the major and branched-chain VFAs in the rumen were increased when soybean curd residue silage was fed to sheep. Similarly, Xu et al. (2001) and Kakihara et al. (2004) reported a higher concentration of total ruminal VFA in sheep fed hay supplemented with soybean curd residue silage than in those fed hay alone. Moreover, the organic acids in soybean curd residue silage (primarily lactic acid) could be converted to acetate, propionate and butyrate in the rumen, as suggested by Gill et al. (1986). Large amount of soluble nitrogenous compounds in soybean curd residue silage increased the NH_3 concentration in the rumen. Similar responses were observed in lactating cows and heifers fed raw soybean curd residue (Chiou et al., 1998; Mlay et al., 2003). The increases in

concentration of VFA and NH_3 in the rumen demonstrated the feasibility of soybean curd residue silage as energy source for ruminant.

Blood metabolites

Examination of the individual blood VFA indicated that the concentration changes for blood acetate and propionate in both groups were reflected by the corresponding changes in the concentrations of these substances in the rumen. Conversely, no significant changes within the sampling time observed for blood butyrate concentration in either group, agreed with those observed from the portal vein by Annison et al. (1957) and from the jugular vein by Evans et al. (1975). In the SCRS-fed sheep, propionate might largely removed by the liver for glucose synthesis, whereas butyrate might be metabolized to ketone bodies in the ruminal epithelium, as suggested by Bergman (1990) and Kristensen (1998). This may explain the reason why the concentrations of propionate and butyrate in the rumen were higher in the SCRS group than Hay group but the concentrations of those acids in the peripheral vein did not differ between the groups. Conversely, there is a trend toward increasing concentration of blood acetate in the SCRS-fed sheep, and it is consistent with the high concentration of acetate in the rumen. This result indicated that in the portal-drained viscera and liver, acetate is less metabolized than propionate and butyrate. The trend toward increased concentration of blood acetate with the SCRS diet suggests a possible increase in acetate availability in the body with feeding soybean curd residue silage.

Although the N intake was enhanced with the inclusion of soybean curd residue silage in the diet, the concentrations of plasma AAs were not significantly affected. It seems that, quantifying the actual protein supply in the ruminant is difficult because

ruminal fermentation alters both the quantity and quality of absorbed dietary AAs. The degradability of dietary protein in the rumen could affect the AA profiles in the blood and almost all of AAs that reached in the intestine are microbial origin in many dietary situations (King et al., 1990; Russell et al., 1992). It is widely understood that NH_3 produced from feed protein degradation in the rumen is used to synthesize microbial protein. On the other hand, elevated concentrations of plasma NH_3 and urea in the SCRS-fed sheep indicated that large quantity of NH_3 from the rumen was absorbed to the blood. This pathway might alter the amount of AA reached and absorbed in the small intestine.

Previous studies on energy balance imply that NEFA concentration in the circulation is reflecting the energy status of the ruminant (Allen et al., 2009). The similar energy intake for sheep fed the SCRS diet and the Hay diet might explain the reason why the plasma NEFA concentrations did not differ between the diets.

Plasma leucine and glucose kinetics

Leucine is the AA that most widely used for measuring protein turnover, precisely because, the first step in the metabolism of Leu is irreversible transamination to α-KIC (Figure 2.6), followed by irreversible decarboxylation (Waterlow, 2006). Therefore, α-KIC is considered as the true precursors of intracellular Leu for protein metabolism study in human (Waterlow, 2006) and animal (Sano et al., 2004). Therefore, although both plasma enrichments of [1-^{13}C]Leu and α-[1-^{13}C]KIC were measured, only the results calculated from α-[1-^{13}C]KIC enrichment in the steady state were discussed.

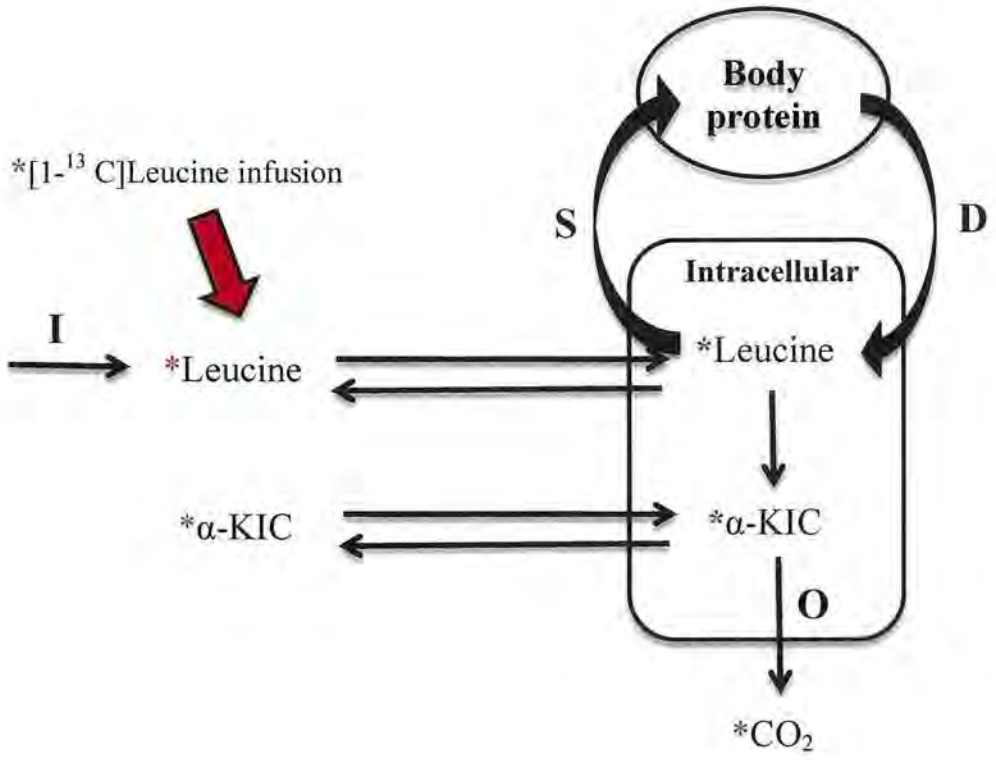


Figure 2.6. Pool model of leucine kinetics. The [1-^{13}C]leucine was administrated to the plasma pool (large arrow) and sampled from plasma. Plasma leucine exchanges with intracellular leucine where metabolism occurs: uptake for protein synthesis (S) or conversion to α-KIC. Oxidation (O) occurs from α-KIC. Unlabeled leucine enters into the free pool via dietary intake (I) or protein degradation (D) into intracellular pools.

Savary-Auzeloux et al. (2003) suggested that the nature of the protein sources could alter the whole body protein turnover in ruminants. Nonetheless, there were no significant changes observed on the plasma LeuTR, as well as WBPS and WBPD when 20% of DM basis of hay was replaced with soybean curd residue silage. Similarly, other studies in sheep also did not find any significant effects on plasma LeuTR, WBPS and WBPD when soybean meal was supplemented to either roughage (Sano et al., 2009) or concentrate based-diet (Sano and Shibasaki, 2011).

In the present study, there was a trend toward increasing plasma glucose concentration with feeding soybean curd residue silage. A similar response was

observed in lambs fed a forage diet supplemented with soybean meal (75 g DM/day) (Ponnampalam et al., 2005). The importance of propionate as a precursor for glucose synthesis in the ruminant liver is well established. In the normal feeding, 53% of whole body glucose is derived from propionate (Seal et al. 1992). Thus, it was expected that a trend toward increasing plasma glucose concentration would be positively correlated with the increased propionate concentration in the rumen of sheep fed the SCRS diet. Moreover, the influence of the propionate supply on the whole body GluTR has been investigated in previous studies through the manipulation in exogenous propionate supply or through the changes in the composition of diets (Veenhuizen et al., 1988; Linington et al., 1998). The findings of the present study were consistent with the results of the previous study by Seal et al. (1992). Those authors found a slightly higher plasma GluTR in steers fed a forage-concentrate diet (2.74 mmol/min) than in steers fed a forage diet (2.18 mmol/min) at similar ME intakes, but the values did not attain statistically significant. Taken together, the present findings showed no detrimental effects on the plasma Leu and glucose kinetics when soybean curd residue silage was fed to sheep.

Conclusion

The current study demonstrated that soybean curd residue silage is a practical source of dietary protein and carbohydrates for sheep. Moreover, soybean curd residue silage provides valuable substrates for N and glucose metabolism without any adverse effects on ruminal fermentation. Utilization of soybean curd residue silage as ruminant feed could reduce the feed scarcity and environmental problem of burning or dumping this high nutritive residue.

Chapter 3

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

Introduction

Recently, economic and environmental concerns have driven investigations into the possibility of using food industry by-products as substitutes for commercial concentrates in animal feeds (Niwa and Nakanishi, 1995; Kajikawa et al., 1996; Chiou et al., 1998; Goh et al., 2002; Kakihara et al., 2004). Previous chapter demonstrated the feasibility of using soybean curd residue silage as an alternative protein source in roughage diets. In other study, Chiou et al. (1998) reported that replacement of the commercial concentrate with raw soybean curd residue in the diet of dairy cows did not adversely affect the milk production and milk constituents. Kakihara et al. (2004) found that lambs fed soybean curd residue silage tended to gain weight faster and had higher concentration of propionate in the rumen than those fed commercial concentrate.

Therefore, it was expected that soybean curd residue silage could replace the commercial concentrate in the diet of sheep without any adverse effects on whole body protein and glucose metabolism because of the high soluble N and fermentable carbohydrate contents in the silage. Therefore, the objective of the second experiment was to determine whether replacing a commercial concentrate with soybean curd residue silage would affect the plasma LeuTR and GluTR by 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

The study was conducted using individual feeding pen and the environmental-controlled house at Faculty of Agriculture, Iwate University. The experimental protocol

and sampling procedure were approved and followed the guidelines established by the Animal Care Committee of Iwate University. Four crossbred (Suffolk x Corriedale) sheep, all of approximately 4 years of age and initially of 56 ± 2 kg of BW were used. Two different dietary treatments, one consisted of 80% mixed hay (orchardgrass and reed canarygrass) and 20% commercial concentrate (CONC diet), and another one (SCRS diet) consisted of 80% mixed hay and 20% soybean curd residue silage containing 15% beet pulp were tested. Soybean curd residue silage used in this second experiment also purchased from Hirakawa Food Co. Ltd. The handling of the silage has been described in Chapter 2. The chemical composition of mixed hay, soybean curd residue silage and commercial concentrate are listed in Table 3.1. The ME intakes for the SCRS diet and CONC diet were formulated slightly above the maintenance level and estimated to be isonitrogenous (NRC, 1985; NARO, 2009) as shown in Table 3.2. The sheep were fed twice daily at 08.30 and 20.30 h and had *ad libitum* access to water.

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. The sheep were weighed at the onset, on day 8, 15 and after the finishing of each dietary treatment. On day 15, the sheep were moved to individual metabolic cages in a controlled-environment room, with an air temperature of 23^{0}C, a relative humidity of 70%, and lighting from 08.00 to 22.00 h. The layout of sampling protocol is illustrated in Figure 3.1.

Table 3.1. Chemical compositions of the mixed hay, soybean curd residue silage and commercial concentrate.

	Mixed hay	Soybean curd residue silage[1]	Commercial concentrate[2]
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
NFC (g/kg DM)	72	477	528
Ash (g/kg DM)	100	80	100
ME (Mcal/kg DM)	1.97	3.32	2.68

[1] Soybean curd residue silage containing 15% beet pulp (Hirakawa Food Co. Ltd., Japan)
[2] Commercial concentrate: formula feed ("α-Beef" made by Chubu Feed Co. Ltd., Japan)

Table 3.2. Diet formulation and intakes of CP and ME of the dietary treatments.

	Treatment[1]	
	CONC diet	SCRS diet
Mixed hay (g/kg $BW^{0.75}$/day)	52.6	48.5
Commercial concentrate (g/kg $BW^{0.75}$/day)	13.2	0
Soybean curd residue silage (g/kg $BW^{0.75}$/day)	0	12.1
CP intake (g/kg $BW^{0.75}$/day)	7.9	7.9
ME intake[2] (Mcal/kg $BW^{0.75}$/ day)	139	135

[1] Treatment: mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is partially replaced with either commercial concentrate (CONC diet) or soybean curd residue silage (SCRS diet) at a ratio of 80:20 on DM basis.
[2] Assumed from NRC (1985).

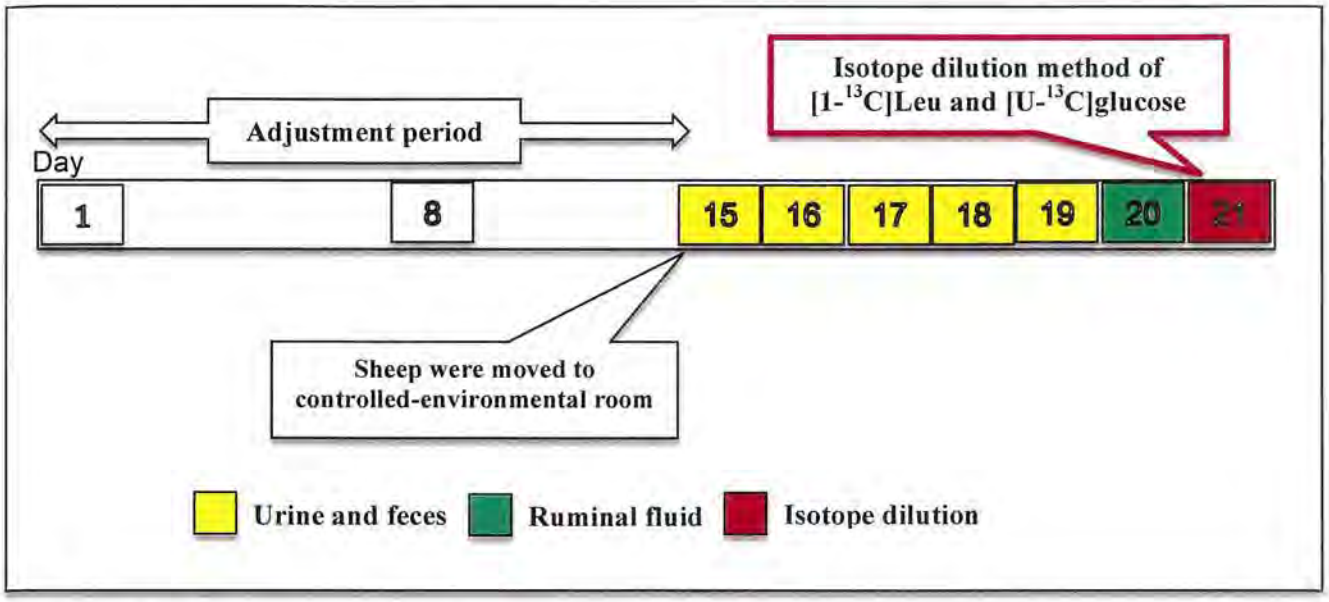


Figure 3.1. The experimental layout showing sampling protocol

Sample collection

Before starting the feeding experiment, samples of the feed were collected and dried in a forced-air oven at 60^{0}C for 48 h 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. All procedures of chemical analysis of feed and the sampling procedure and treatment of urine, feces and ruminal fluid have been described in detail in Chapter 2.

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 h, between 3 and 7 h after the morning feeding. The concentrations of saline solution and infusion rates of [1-^{13}C]Leu and [U-^{13}C]glucose, and blood sampling procedures were same as those described in Chapter 2.

Sample analysis, calculation and statistical analysis

The analytical procedures of sample analysis, calculations (LeuTR, GluTR, WBPD and WBPD) and statistical analysis of the data have been described in detail in the Chapter 2.

Results

Silage quality and nitrogen balance

The fermentative characteristics of soybean curd residue silage are shown in Table 3.3. The pH value was 4.14, and organic acids, such as lactic acid, acetate, propionate and butyrate, were detected.

Table 3.3. Fermentative characteristics of the soybean curd residue silage

Item	Amount
Moisture (g/kg)	690
pH	4.14
Lactic acid (g/kg FM)	16.9
Acetate (g/kg FM)	1.1
Propionate (g/kg FM)	0.03
Butyrate (g/kg FM)	0.003
VBN (g/kg total N)	68
Flieg point	100
V-score	98

The BW gain was similar (P=0.21) between sheep fed the SCRS diet and those fed the CONC diet (Table 3.4). 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 CONC 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 CONC diet. The N absorption and digestibility did not differ (P=0.11 and P=0.14, respectively) between the two diets. The amount of N retained in sheep fed the SCRS diet and CONC diet were similar (P=0.42).

Table 3.4. The effect of the source of N on the BW gain and N balance in sheep[1]

Items	Treatment[2]		SEM[3]	*P* value
	CONC diet	SCRS diet		
BW gain (kg/day)	0.12	0.15	0.03	0.21
N intake (g/kg $BW^{0.75}$/day)	1.27	1.28	0.005	0.05
Fecal N (g/kg $BW^{0.75}$/day)	0.48	0.45	0.03	0.17
Urinary N (g/kg $BW^{0.75}$/day)	0.48	0.55	0.07	0.05
N absorption (g/kg $BW^{0.75}$/day)	0.79	0.83	0.01	0.11
N retention (g/kg $BW^{0.75}$/day)	0.31	0.28	0.02	0.42
N digestibility (%)	62.5	65.0	1.0	0.14

[1] Values represent the means for n = 4.
[2] Treatment, mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is partially replaced with either commercial concentrate (CONC diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on DM basis.
[3] SEM, standard error of the mean.

Ruminal fermentation characteristics

The observed changes in the ruminal characteristics during the sampling period are provided in Figure 3.2. The effects of the two diets on the ruminal pH, NH_3 and VFA concentrations, represented as the mean value of four sheep in three sampling periods (0, 3, and 6 h after feeding), are shown in Table 3.5. 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 to have a higher ruminal pH than those fed the CONC diet (P=0.05). The pH values under both treatments were above 6.8 prior to feeding, and the values dropped to approximately 6.7 at 3 h after feeding. Comparable NH_3 concentrations were found for both groups (P=0.22); the NH_3 concentrations were increased at 3 h 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 CONC diet, resulting in a lower acetate:propionate ratio (P=0.03) for the SCRS than CONC diet. The butyrate concentration was lower (P=0.02) for the SCRS diet compared to the CONC 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 h after feeding and eventually returned toward previous values. The isobutyrate and isovalerate concentrations were gradually decreased at 6 h after feeding.

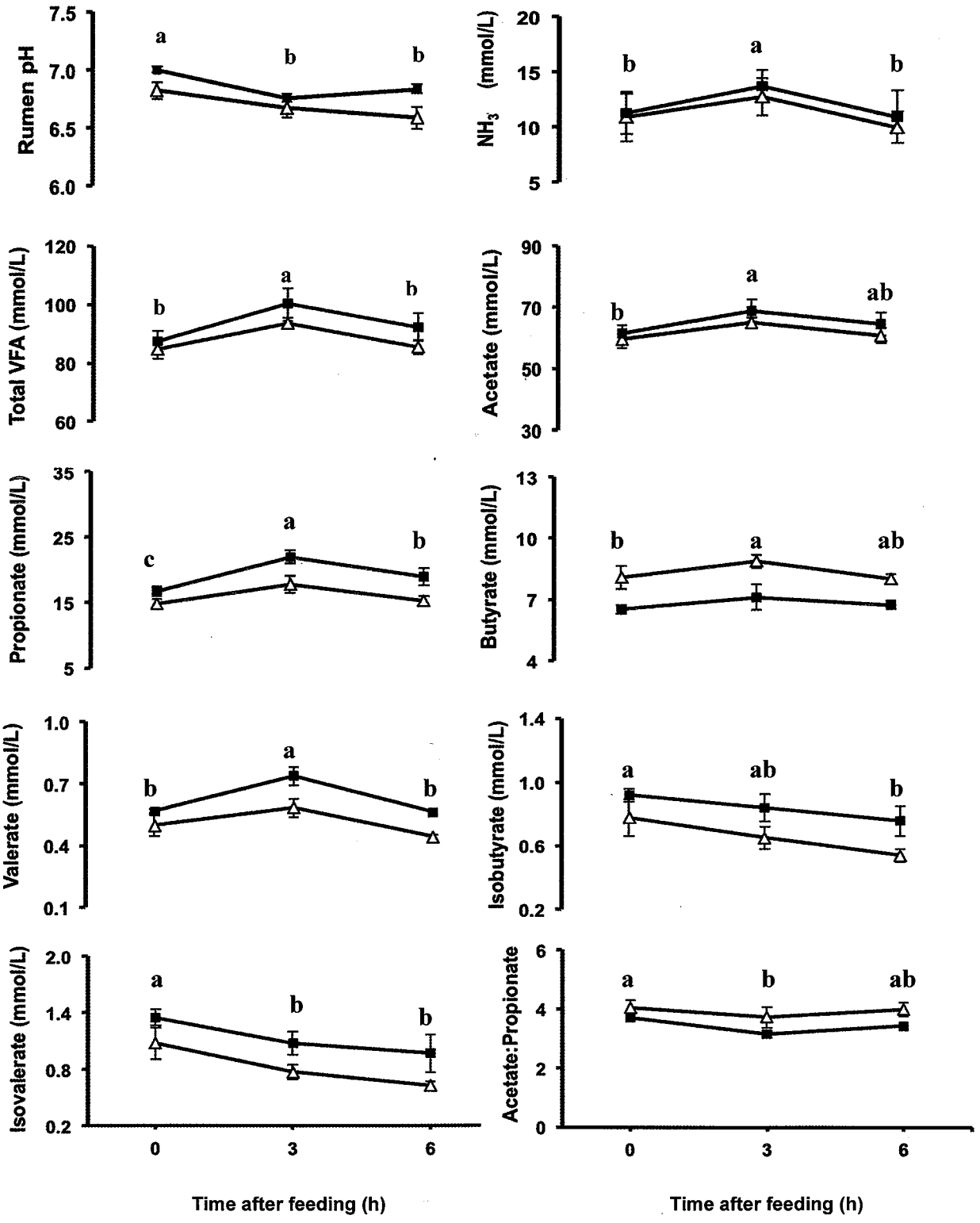


Figure 3.2. Time course changes of ruminal characteristics in sheep fed the SCRS diet (■) and the CONC diet (Δ). Values are expressed as mean ± SEM for n = 4. Different letters (a, b, c) indicate significant different ($P<0.05$) between time after feeding.

Table 3.5. The effects of the source of N on the ruminal pH, NH_3 and VFA in sheep[1]

Item	Treatment[2]		SEM[3]	*P* value		
	CONC diet	SCRS diet		Treatment	Time	Treatment x Time
pH	6.69	6.87	0.03	0.05	0.008	0.19
NH_3 (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
Butyrate	8.3	6.8	0.4	0.02	0.07	0.68
Isobutyrate	0.7	0.8	0.1	0.05	0.01	0.56
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	3.9	3.4	0.2	0.03	0.008	0.63

[1] Values represent the means for n = 4.
[2] Treatment, mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is partially replaced with either commercial concentrate (CONC diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on DM basis.
[3] SEM, standard error of the mean.

Plasma metabolite concentrations

The plasma AA, urea and NEFA concentrations determined at the pre-isotope infusion period at 3 h after feeding are presented in Table 3.6. 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 CONC diet; the concentrations of other AA were comparable between the diets. The plasma NH_3 and urea concentrations were higher ($P=0.03$ and $P=0.02$) in the sheep fed the SCRS than those fed the CONC diet, whereas plasma NEFA concentration did not differ ($P=0.38$) between the diets.

Plasma leucine and glucose kinetics

The plasma Leu and α-KIC concentrations and plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichments were at constant level during the last 2 h of isotope infusion (Figure 3.3). The plasma Leu and α-KIC concentrations did not differ ($P=0.18$ and $P=0.13$, respectively) between the diets (Table 3.7). The sheep fed the SCRS diet had similar ($P=0.74$) plasma LeuTR with those of sheep fed the CONC diet. The WBPS and WBPD were comparable ($P=0.23$ and $P=0.24$) between the diets. The plasma glucose concentration and [U-^{13}C]glucose enrichment were essentially constant during the last 2 h of the isotope infusion (Figure 3.4). The plasma glucose concentration and GluTR did not differ ($P=0.68$ and $P=0.27$, respectively) between the two diets (Table 3.7).

Table 3.6. The effects of the source of N on the plasma AA, NH_3, urea and NEFA concentrations at pre-infusion of isotope in sheep[1]

Item	Treatment[2]		SEM[3]	*P* value
	CONC 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
NH_3 (μmol/L)	191	221	7	0.03
Urea (mmol/L)	6.21	7.80	0.2	0.02
NEFA (μEq/L)	105	125	11	0.38

[1] Values represent the means for n = 4.
[2] Treatment, mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is partially replaced with either commercial concentrate (CONC diet) or soybean curd residue silage (SCRS diet), at a ratio of 80:20 on DM basis.
[3] SEM, standard error of the mean.

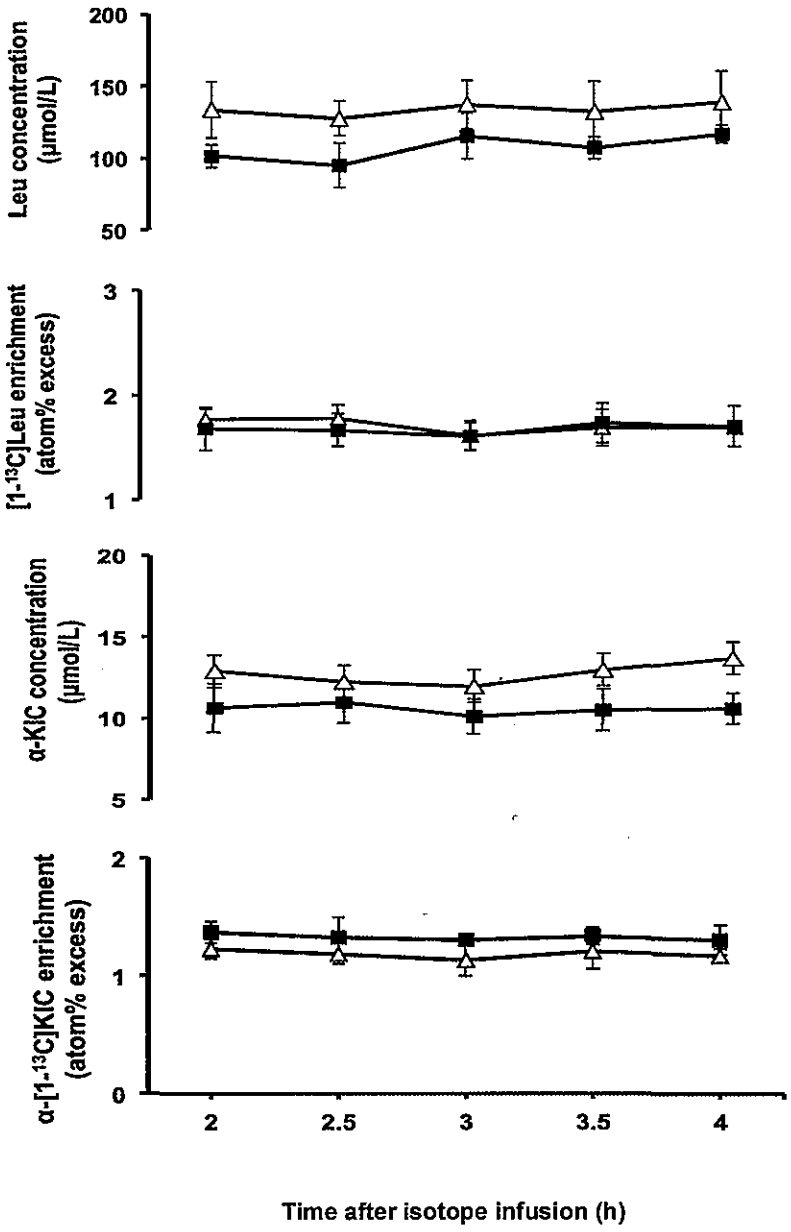


Figure 3.3. Time course changes of plasma Leu and α-KIC concentrations, plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC enrichments during the last 2 h of isotope infusion in sheep fed the SCRS diet (■) and the CONC diet (Δ).Values are expressed as mean ± SEM for n = 4.

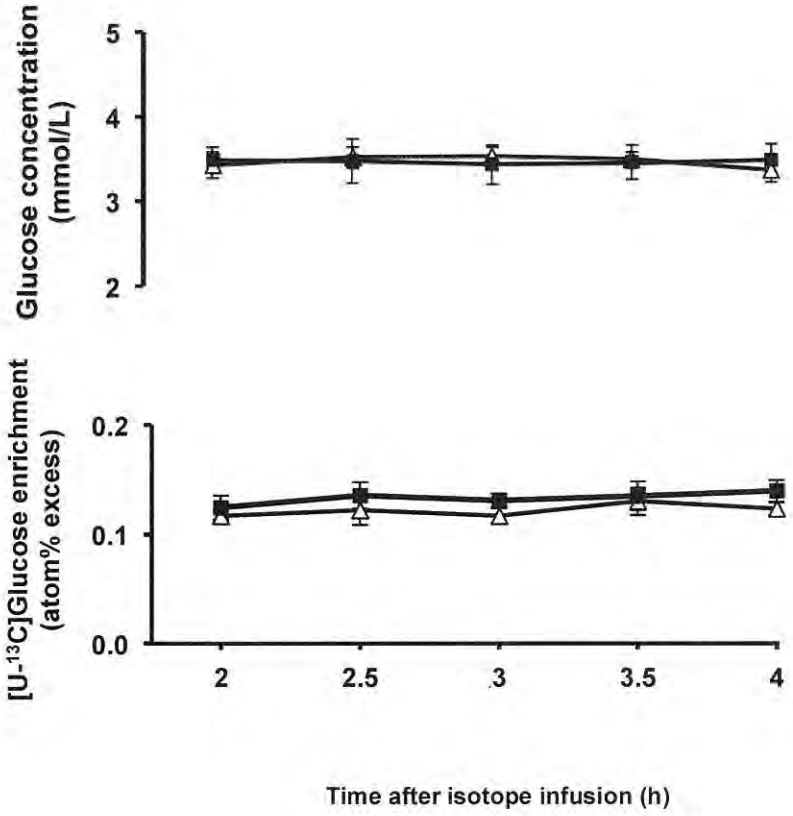


Figure 3.4. Time course changes of plasma glucose concentration and [U-^{13}C]glucose enrichment during the last 2 h of isotope infusion in sheep fed the SCRS diet (■) and the CONC diet (Δ). Values are expressed as mean ± SEM for n = 4

Table 3.7. The effects of the source of N on the plasma Leu and glucose kinetics in sheep[1]

Item	Treatment[2]		SEM[3]	*P* value
	CONC diet	SCRS diet		
Leu kinetics				
Leu concentration (μmol/L)	135	107	13	0.18
α-KIC concentration (μmol/L)	12.8	10.3	0.5	0.13
LeuTR (μmol/kg $BW^{0.75}$/h)	521	507	23	0.74
WBPS (g/kg $BW^{0.75}$/day)	21.7	20.7	1.1	0.23
WBPD (g/kg $BW^{0.75}$/day)	19.8	18.8	1.2	0.24
Glucose kinetics				
Glucose concentration (mmol/L)	3.34	3.40	0.18	0.68
GluTR (mmol/kg $BW^{0.75}$/h)	2.24	2.14	0.09	0.27

[1] Values represent the means for n = 4.
[2] Treatment, mixed hay (orchardgrass and reed cannarygrass) as the basal diet which is partially replaced with either commercial concentrate (CONC diet) or soybean curd residue silage (SCRS diet) at a ratio of 80:20 on DM basis.
[3] SEM, standard error of the mean.

Discussion

Silage quality

The silage was well preserved, as indicated by the low pH, high lactic acid content, high Flieg point and V-score. The chemical compositions and organic acid concentrations were comparable with those observed in previous experiment (Chapter 2). The lactic acid concentration in the silage was similar to that found by Amaha et al. (1996), but it was higher than that found by Xu et al. (2001). The NDF content of soybean curd residue silage (150 g/kg DM) was comparable to those reported in raw

soybean curd residue (145 g/kg DM) by O'Toole (1999), but it was lower than the NDF content of commercial concentrate (207 g/kg DM).

Nitrogen balance

Previous studies (Oltjen and Putnam, 1966; Clifford and Tillman, 1968; Prior, 1976; Knaus et al., 2002) have shown that inclusion soy protein in the diet of ruminants enhanced 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 soybean curd residue silage did not affect the amount of N absorbed and retained, whereas it did slightly affect the urinary N excretion. Hence, current findings demonstrated the feasibility of replacing commercial concentrate with soybean curd residue silage to maintain a positive N balance.

Ruminal fermentation 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. Thus, replacing the commercial concentrate with soybean curd residue silage did not impair ruminal fermentation; a similar response observed by Xu et al. (2001). The SCRS diet and the CONC diet in the present study were approximately isonitrogenous and isoenergetic; thus, similar N compounds should be available for NH_3 production in the rumen. This finding is consistent with the results of Ipharraguerre et al. (2005) who

reported that the concentration of NH_3 in the rumen fluid only affected by the level of dietary protein intake.

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). Although the NFC content in soybean curd residue silage (477 g/kg DM) is less than concentrate (528 g/kg DM), the organic acids in the silage, mainly lactic acid might be contributed to the higher propionate concentration in the rumen of sheep fed the SCRS diet than those fed the CONC diet. Previously, Beauchemin (1991) suggested that NDF concentration in a ruminant diet is positively correlated with the amount of VFA produced in the rumen. Nonetheless, the lower NDF content for soybean curd residue silage (150 g/kg DM) compared to the commercial concentrate (207 g/kg DM) did not alter the total VFA concentration in the rumen. Present findings demonstrated that replacing the commercial concentrate with soybean curd residue did not significantly influence the total VFA concentration, indicating similar efficiencies of energy-source production for both diets.

Plasma leucine kinetics

The primary source of dietary protein affected the plasma AA concentrations in sheep (Schelling et al., 1967). 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 plasma AA levels were most likely due to proteolytic activity during the fermentation process, which could alter the composition of AA in the silage (Ohshima and McDonald, 1978). Another possibilities

that might affect the concentration of AA in the plasma are the type of protein and the digestibility in the rumen. Because both factors could alter the amount of AA absorbed in the small intestine, as discussed previously in Chapter 2. Indeed, although the ruminal NH_3 concentrations within the sampling periods (0, 3, and 6 h after feeding) did not differ between the two diets, higher concentrations of plasma NH_3 and urea were observed in sheep fed the SCRS diet than those fed the CONC diet, indicated that large amount of NH_3 in the rumen of SCRS-fed sheep was absorbed to blood. Furthermore, the N excretion via the urine tended to increase over time. Although the sheep fed the SCRS diet in our study exhibited lower concentrations of certain plasma AAs, replacing the commercial concentrate with soybean curd residue silage did not induce an AA imbalance, based on the positive N balance and similar N retention for both of the diets.

The plasma LeuTR observed in current experiment was comparable to the previous experiment (Chapter 2), and also comparable to those in another 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 also comparable to those from another 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 the current experiment did not alter either the plasma Leu or glucose kinetics of the sheep. Nielsen et al. (1994) suggested that the use of different protein sources influence the turnover rate of protein. Report on the protein turnover of soybean-based diets measured by isotope dilution technique in ruminant is limited, but there are some studies conducted in pigs and human (Deutz et al., 1998; Bos et al., 2003). They reported that the AAs from soybean-based diets were highly catabolized in the body and resulted in lower protein turnover compared with the diet containing casein.

Nonetheless, in current experiment, plasma LeuTR, WBPS and WBPD between sheep fed the soybean curd residue silage and commercial concentrate were comparable. The inconsistency with current study possibly related to the species, treatment and diet used. Moreover, the dietary treatments in current experiment 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 ME intake in sheep (Liu et al., 1995) and cows (Lapierre et al., 2002). Similarly, Sano et al. (2009) reported comparable plasma LeuTR, WBPS and WBPD for different protein sources (urea and soybean meal) in sheep fed the isoenergetic and isonitrogenous diets.

Plasma glucose kinetics

The GluTR is influenced by several factors, including the energy intake and supply of gluconeogenetic substrate to the liver (Ortigues-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, the SCRS diet enhanced the availability of glucogenic substrate and resulted in the similar plasma GluTR with the CONC diet. Hence, present findings demonstrated that replacing commercial concentrate with soybean curd residue silage in the diet of sheep did not adversely affect plasma Leu and glucose kinetics. Furthermore, the comparable results for the plasma Leu and glucose kinetics indicated that soybean curd residue silage could provide sufficient nutrient as well as commercial concentrate, due to its abundant soluble N and fermentable carbohydrate compounds. Taken together, similar plasma

Leu and glucose kinetics responses could be achieved by replacing commercial concentrate with soybean curd residue silage in the same amount of ME intake.

As discussed in the previous chapter, the circulating plasma NEFA concentration was closely 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 and Kuhlemeier, 1966). Hence, the comparable plasma NEFA concentrations indicated comparable nutritional status between sheep fed the SCRS and CONC diet.

Conclusion

Present findings demonstrated that replacing commercial concentrate with soybean curd residue silage in the diet of sheep did not impair ruminal fermentation, and enhanced the glucogenic propionate concentration. The inclusion of soybean curd residue silage in the diets of sheep could ensure a positive N balance and resulted in similar plasma Leu 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.

Chapter 4

The Effects of Dietary Level of Soybean Curd Residue Silage on Ruminal Characteristics and Plasma Leucine, Glucose and Acetate Kinetics in Sheep Fed Roughage Diets

Introduction

Low productivity in ruminants is mostly attributed to inefficient protein supply. However, protein supplementation can be costly. It has been proved that soybean curd residue silage could replace commercial concentrate (Chapter 3), but the appropriate feeding level of this by-product must be identified. Goh et al. (2002) reported that sheep fed a 40% concentrate containing soybean curd residue gained faster than those that received lower quantities. In a fattening study, soybean curd residue supplementation (30%) to the diet of goats increased the fatty acid content in the carcass (Hirayama et al., 2002). Similarly, Niwa and Nakanishi (1995) reported an increase in the fatty acid content of the carcasses of pigs supplemented with 50% soybean curd residue silage. It is widely understood that acetate is the most important metabolite in the fatty acid and carbohydrate metabolism in ruminants, whereas propionate is the major precursor in gluconeogenesis. Both acetate and propionate concentrations in the rumen were increased with soybean curd residue silage (Chapter 2). Huntington et al. (2006) suggested that increasing glucogenic substrates availability could increase hepatic gluconeogenesis and glucose entry rates. However, to our knowledge, there is no information available regarding the effect of amounts of soybean curd residue silage consumption on the turnover rates of protein, glucose and acetate in ruminants.

Therefore, the third experiment was conducted to investigate the effects of dietary level of soybean curd residue silage on the kinetics of plasma Leu, glucose and acetate in sheep using the primed-continuous infusion method with [1-^{13}C]Leu, [U-^{13}C]glucose and [1-^{13}C]Na acetate. The ruminal fermentation characteristics and N balance were also determined.

Materials and methods

Animals, diets and management

The experimental protocols for animal care and use were in accordance with the guidelines established by the Iwate University Animal Care Committee. Six crossbred (Suffolk × Corriedale) sheep with an initial BW of approximately 39 ± 1 kg were used. Sheep were fed three diets differing in the amount of soybean curd residue silage: 100% mixed hay (SCRS-0, as a control), 80% mixed hay plus 20% soybean curd residue silage (SCRS-20), and 60% mixed hay plus 40% soybean curd residue silage (SCRS-40) on a DM basis. The soybean curd residue silage used contained 15% beetpulp and it was purchased from Hirakawa Food Co. Ltd. The ensiling period and handling of the silage were same as those described in the Chapter 2. The chemical components of the mixed hay and soybean curd residue silage are listed in Table 4.1. The ME intakes for all dietary treatments were formulated slightly above the maintenance level (NRC, 1985; NARO, 2009), as shown in Table 4.2. The sheep were fed twice daily, at 08.30 and 20.30 h, and had access to water *ad libitum*.

The experiment followed a 3 × 3 Latin square design with a period of 21 days, composed of 14 days of adaptation to the diet and 7 days of sample collection. During the adaptation period, the sheep were housed in individual pens. 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 h. The schematic layout of sampling protocol is shown in Figure 4.1.

Table 4.1. Chemical compositions of soybean curd residue silage and dietary treatments.

	Treatment[1]			Soybean curd residue silage
	SCRS-0 (Mixed hay)	SCRS-20	SCRS-40	
DM (g/kg)	900	784	668	320
CP (g/kg DM)	106	128	149	214
EE (g/kg DM)	34	49	64	110
NDF (g/kg DM)	690	578	469	137
NFC (g/kg DM)	70	162	254	531
Ash (g/kg DM)	100	96	92	80

[1] Treatment, mixed hay of orchardgrass and reed canarygrass was partially replaced with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

Table 4.2. Diet formulation and intakes of CP and ME of the dietary treatments.

	Treatment[1]		
	SCRS-0	SCRS-20	SCRS-40
Mixed hay (g/kg $BW^{0.75}$/day)	62.2	43.6	28.6
Soybean curd residue silage (g/kg $BW^{0.75}$/day)	0	10.4	19.5
CP intake (g/kg $BW^{0.75}$/day)	6.6	7.1	7.5
ME intake[2] (Mcal/kg $BW^{0.75}$/day)	122	120	119

[1] Treatment, mixed hay of orchardgrass and reed canarygrass was partially replaced with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.
[2] Assumed from NRC (1985).

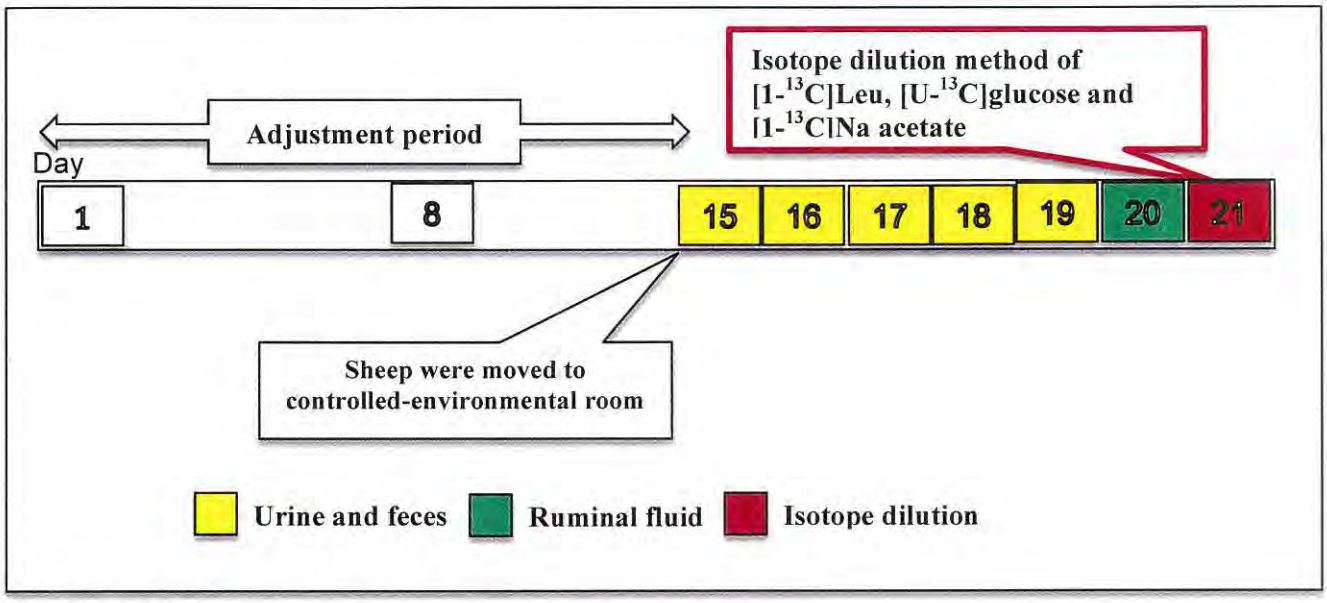


Figure 4.1. The experimental layout showing sampling protocol.

The similar isotope dilution procedure as previous experiments (Chapter 2 and 3) was conducted on day 21 of each treatment. The isotope [1-^{13}C]Na acetate was used in addition to [1-^{13}C]Leu and [U-^{13}C]glucose, to determine the plasma Leu, glucose and acetate kinetics simultaneously. Two catheters, one for isotope infusion and another one for blood sampling, were inserted into the right and left jugular veins, respectively. The catheters were filled with sterile 3.8% trisodium citrate (w/v). Blood samplings were performed without any noticeable stress to the sheep.

Ruminal fluid, urine and feces collection and chemical analysis

The sampling method and chemical analysis for feed, urine, feces and ruminal fluid were the same as those described in Chapter 2.

Application of isotope dilution techniques

The isotope dilution procedures used to measure the plasma Leu, glucose and acetate kinetics were performed simultaneously on day 21. The primed-continuous infusion of [1-^{13}C]Leu, [U-^{13}C]glucose and [1-^{13}C]Na acetate was conducted over a period of 4 h, between 3 and 7 h after the morning feeding. A saline tracer solution containing 7.2 μmol/kg $BW^{0.75}$ of [1-^{13}C]Leu, 2.9 μmol/kg $BW^{0.75}$ of [U-^{13}C]glucose and 87 μmol/kg $BW^{0.75}$ of [1-^{13}C]Na acetate (1-^{13}C, 99 atom% excess ^{13}C; Cambridge Isotope Laboratories, USA) was injected into the right jugular catheter as a priming dose. This tracer solution was then continuously infused through the same catheter using a multichannel peristaltic pump (AC-2120, Atto, Japan) at rates of 7.2, 2.9 and 87 μmol/ kg $BW^{0.75}$/h for [1-^{13}C]Leu, [U-^{13}C]glucose and [1-^{13}C]Na acetate, respectively. The infusion rate of the tracer solution was recorded every 30 min throughout the infusion period. Blood samples (6 mL) were collected from the left jugular catheter immediately before the priming injection and at 30-min intervals during the last 2 h of isotope infusion (5 to 7 h after feeding). The blood samples were placed in heparinized tubes and stored on ice. The plasma from the blood sample was separated by centrifugation at 8,000 × *g* for 10 min at 4°C and then stored at -30°C until further analysis.

Chemical analysis of plasma metabolites and isotope enrichments

The procedure of chemical analysis to determine the concentrations of plasma Leu and α-KIC, the enrichments of the plasma [1-^{13}C]Leu and α-[1-^{13}C]KIC, and the concentrations of plasma metabolites (AA, NH_3, urea, NEFA and glucose) have been described in detail previously (Chapter 2).

The plasma [1-^{13}C]acetate enrichment and the concentrations of plasma acetate and lactate were determined using a selected ion monitoring system with GC-MS as described by Moreau et al. (2003). Briefly, the plasma (1 mL) was deproteinized by adding 1 mL of 4% SSA and 100 μL of an internal standard containing 0.5 mmol/L of 2-ethylbutyric acid and 0.5 mmol/L of 4-methylvaleric acid. After centrifugation at 12,000 × *g* for 10 min at 0°C, the supernatant was collected in a screw-cap glass tube and acidified by adding 25 μL of 37% HCl. A double extraction procedure was performed with diethyl ether, and the two phases were then separated by centrifugation at 1,000 × *g* for 3 min at 0°C. The upper organic layer was transferred into a new screw-cap glass tube, and an additional ethyl extraction was performed on the aqueous layer. After the complete recovery of the organic layer, 100 μL of the organic sample was placed in new screw-cap glass tube and added with 20 μL of MTBSTFA. The following ions were evaluated: m/z 117 and 118 for the [1-^{13}C]acetate enrichment, m/z 117 for acetate concentration and m/z 261 for lactate concentration.

Calculation and statistical analysis

The turnover rate of plasma acetate (AceTR) was calculated according to Wolfe and Chinkes (2005).

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

where I is the infusion rates of [1-^{13}C]Na acetate and E is the isotope enrichment of plasma [1-^{13}C]acetate during the steady state.

The WBPS and WBPD were calculated according to Schroeder et al. (2006) and Harris et al. (1992) and all data were analyzed using the MIXED procedure of SAS (1996) as described previously (Chapter 2).

Results

Silage quality, body weight gain and nitrogen balance

The silage pH was 4.06 and the silage had a high lactic acid content (Table 4.3). Other organic acids, such as acetate, propionate and butyrate, were also detected in trace quantities.

Table 4.3. The fermentative characteristics in the soybean curd residue silage

Item	Amount
Moisture (g/kg)	680
pH	4.06
Lactic acid (g/kg FM)	13.0
Acetate (g/kg FM)	1.3
Propionate (g/kg FM)	0.02
Butyrate (g/kg FM)	0.001
VBN (g/kg total N)	40
Flieg point	100
V-score	98

Inclusion of soybean curd residue silage in the diet tended to increase the BW gain (P=0.06) (Table 4.4). As expected, the N intake increased (P<0.0001) when the level of soybean curd residue silage in the diets increased. The fecal N excretion

decreased (P<0.001) and urinary N excretion increased (P<0.001) with increasing dietary silage content in the diets. Increasing the dietary levels of soybean curd residue silage resulted in an increase (P<0.001 and P=0.001) in the N absorption and digestibility, but the N retention did not significantly affected (P=0.23).

Table 4.4. The effects of dietary level of soybean curd residue silage on BW gain and N balance in sheep[1]

Item	Treatment[2]			SEM	P value
	SCRS-0	SCRS-20	SCRS-40		
BW gain (kg/day)	0.06	0.10	0.11	0.01	0.06
N intake (g/kg $BW^{0.75}$/day)	1.05[c]	1.13[b]	1.20[a]	0.01	<0.0001
Fecal N (g/kg $BW^{0.75}$/day)	0.42[a]	0.31[b]	0.26[c]	0.02	<0.001
Urinary N (g/kg $BW^{0.75}$/day)	0.25[c]	0.42[b]	0.50[a]	0.02	<0.001
N absorption (g/kg$BW^{0.75}$/day)	0.63[c]	0.82[b]	0.94[a]	0.01	<0.001
N retention (g/kg $BW^{0.75}$/day)	0.38	0.40	0.44	0.03	0.23
N digestibility (%)	60.2[c]	73.4[b]	78.4[a]	1.1	0.001

[a,b,c] Means within a row with different letters differ (P<0.05)

[1] Values represent means for n = 6

[2] Treatment, partial replacement of mixed hay with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

Ruminal fermentation characteristics

The ruminal pH values were approximately 7.0 prior to feeding, and the values dropped to approximately 6.8 at 3 h after feeding and remained constant at 6 h after feeding (Fig. 1). The patterns of NH_3, acetate, propionate and butyrate concentrations in the response to feeding were similar for all groups, in which the concentrations of NH_3 and those acids were increased gradually from before feeding to 3 h after feeding then decreased gradually afterward. Increased soybean curd residue silage level in the diets did not influence (P=0.85) the ruminal pH (Table 5). The ruminal NH_3 concentrations were increased (P=0.001) with increasing amount of soybean curd residue silage in the diets. Inclusion of soybean curd residue silage in the diets had significant influence (P=0.02; P=0.02 and P=0.02) in the concentrations of ruminal total VFA, acetate and propionate. The ruminal total VFA, acetate and propionate concentrations were higher (P<0.05) in the SCRS-20 and SCRS-40 groups than in the SCRS-0 group. However, no differences between the SCRS-20 and SCRS-40 groups were observed in the concentrations of those acids in the rumen (P≥0.05). The ruminal butyrate concentrations were not influenced (P=0.15) by the treatments, whereas the valerate concentration increased (P=0.002) with increasing amount of soybean curd residue silage in the diets. The ruminal isobutyrate concentration tended (P=0.08) to increase with increasing dietary silage content in the diets, whereas the ruminal isovalerate concentrations remained unchanged (P=0.43). The dietary level of soybean curd residue silage tended to affect (P=0.06) the molar ratio of acetate to propionate.

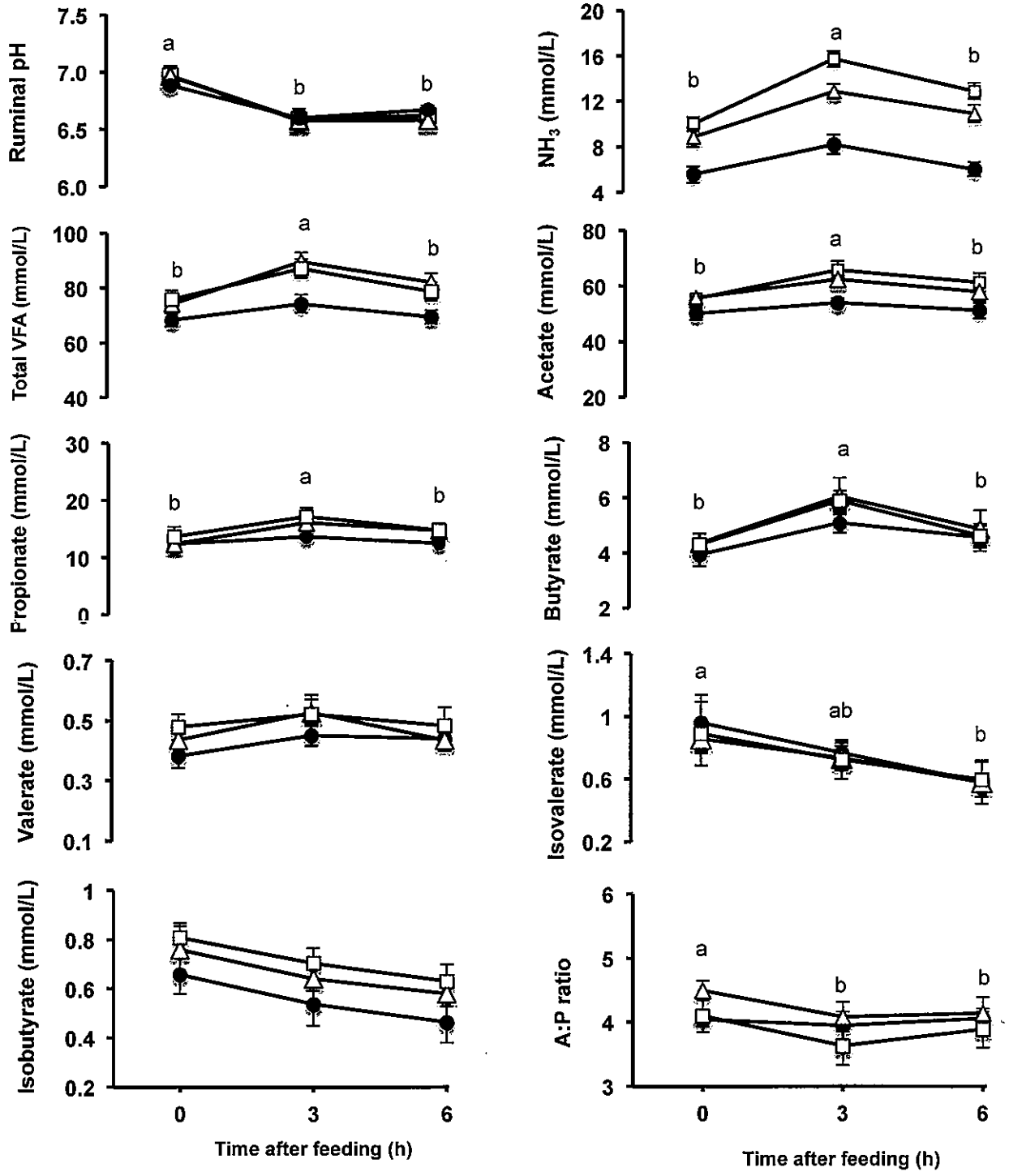


Figure 4.2. Time course changes of the ruminal characteristics in sheep fed SCRS-0 (●), SCRS-20 (△) and SCRS-40 (□). The values are expressed as the mean ± SEM for n=6. Different letters (a, b) indicate significant difference ($P<0.05$) between times after feeding.

Table 4.5. The effects of dietary level of soybean curd residue silage on ruminal pH, and the concentrations of NH_3 and VFA in sheep[1]

Item	Treatment[2]			SEM	*P* value
	SCRS-0	SCRS-20	SCRS-40		
pH	6.74	6.61	6.60	0.05	0.85
NH_3 (mmol/L)	6.6[c]	10.9[b]	12.9[a]	0.4	0.001
Total VFA (mmol/L)	71.0[b]	81.6[a]	80.7[a]	2.3	0.02
Individual VFA concentrations (mmol/L)					
Acetate	51.7[b]	60.4[a]	58.6[a]	2.3	0.02
Propionate	12.9[b]	14.3[a]	15.2[a]	0.5	0.02
Butyrate	4.6	5.0	4.9	0.4	0.15
Valerate	0.42[c]	0.47[b]	0.50[a]	0.09	0.002
Isobutyrate	0.55	0.66	0.71	0.12	0.08
Isovalerate	0.77	0.72	0.74	0.14	0.43
Acetate:propionate	4.0	4.2	3.9	0.1	0.06

[a,b,c] Means within a row with different letters differ ($P<0.05$)

[1] Values represent means for n = 6

[2] Treatment, partial replacement of mixed hay with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

Plasma metabolite concentrations

Concentrations of plasma threonine, valine, methionine, phenylalanine, glutamic acid, glycine, alanine, tyrosine, arginine and proline did not differ ($P \geq 0.10$) among treatments. Whereas plasma isoleucine, Leu, histidine, lysine, serine, asparagine and glutamine were present at lower concentrations ($P<0.05$) in the SCRS-20 and SCRS-40 groups than in the SCRS-0 group (Table 4.6). The plasma NH_3 and urea concentrations increased ($P<0.0001$ and $P=0.002$) with increasing amounts of soybean curd residue silage in the diet. The inclusions of soybean curd residue silage in the diets affect ($P=0.01$) the concentration of plasma lactate. The plasma lactate concentrations were higher ($P<0.05$) in the SCRS-20 and SCRS-40 groups than in the SCRS-0 group, and the concentration between SCRS-20 and SCRS-40 groups were similar ($P \geq 0.05$). The plasma NEFA concentrations did not differ among the treatments ($P=0.85$).

Plasma leucine kinetics

The plasma concentrations of Leu and α-KIC and the isotope enrichment of α-[1-^{13}C]KIC were stable over the last 2 h of isotope infusion (Figure 4.3). The concentrations of plasma Leu and α-KIC were similar ($P=0.90$ and $P=0.34$) among all groups (Table 4.7). Increasing the dietary levels of soybean curd residue silage did not significantly influence the plasma LeuTR, WBPS and WBPD ($P=0.17$, $P=0.47$ and $P=0.58$, respectively).

Table 4.6. Plasma AA, NH_3, urea, lactate and NEFA concentrations in sheep[1]

Item	Treatment[2]			SEM	*P* value
	SCRS-0	SCRS-20	SCRS-40		
Essential AA (μmol/L)					
Threonine	200	190	189	10	0.50
Valine	284	257	251	13	0.12
Methionine	27	21	20	4	0.11
Isoleucine	103[a]	87[b]	83[b]	4	0.03
Leucine	143[a]	123[b]	113[b]	5	0.02
Phenylalanine	62	53	52	4	0.13
Histidine	63[a]	53[b]	54[b]	2	0.02
Lysine	143[a]	108[b]	106[b]	10	0.02
Non-essential AA (μmol/L)					
Serine	173[a]	116[b]	125[b]	11	0.01
Asparagine	106[a]	83[b]	90[b]	7	0.03
Glutamic acid	53	49	47	4	0.32
Glutamine	334[a]	259[b]	262[b]	20	0.003
Glycine	581	502	545	33	0.16
Alanine	201	196	201	11	0.78
Tyrosine	79	67	67	6	0.23
Arginine	175	140	149	14	0.16
Proline	114	93	98	9	0.14
NH_3 (μmol/L)	123[c]	206[b]	218[a]	6	<0.0001
Urea, mmol/L	4.40[c]	5.72[b]	6.63[a]	0.1	0.002
NEFA (μEq/L)	146	144	140	13	0.85
Lactate (mmol/L)	0.33[b]	0.48[a]	0.47[a]	0.04	0.01

[a,b,c] Means within a row with different letters differ ($P<0.05$)

[1] Values represent means for n = 6

[2] Treatment, partial replacement of mixed hay with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

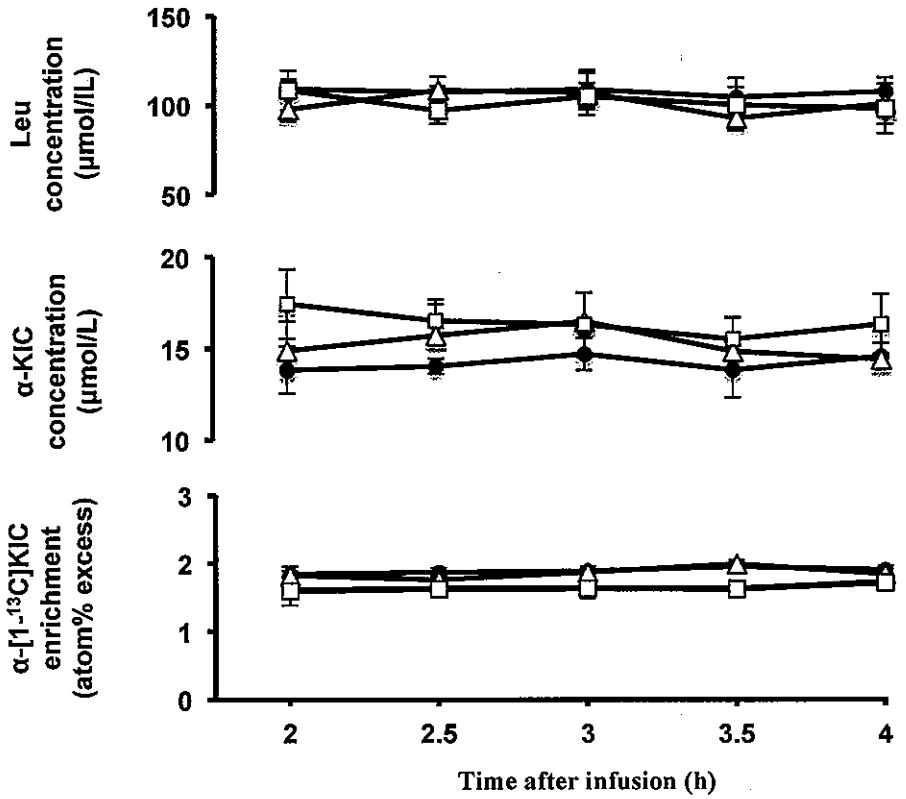


Figure 4.3. Plasma Leu and α-KIC concentrations and plasma α-[1-^{13}C]KIC enrichment in sheep fed SCRS-0 (●), SCRS-20 (△) and SCRS-40 (□).

Table 4.7. The effects of dietary level of soybean curd residue silage on plasma Leu kinetics in sheep[1]

Item	Treatment[2]			SEM	*P* value
	SCRS-0	SCRS-20	SCRS-40		
Leu Concentration (μmol/L)	107	105	106	4	0.90
α-KIC Concentration (μmol/L)	14.5	15.6	16.0	1.0	0.34
LeuTR (μmol/kg $BW^{0.75}$/h)	469	516	572	21	0.17
WBPS (g/kg $BW^{0.75}$/day)	20.8	22.0	22.3	2.1	0.47
WBPD (g/kg $BW^{0.75}$/day)	18.4	19.4	19.5	2.0	0.58

[1] Values represent means for n = 6

[2] Treatment, partial replacement of mixed hay with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, at ratio 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

Plasma glucose and acetate kinetics

The plasma glucose concentration and [U-^{13}C]glucose enrichment were constant during the last 2 h of isotope infusion, as shown in Figures 4.4. The inclusions of soybean curd residue silage in the diets affect (P=0.03) the concentration of plasma glucose (Table 4.8). The plasma glucose concentrations were higher (P<0.05) in the SCRS-20 and SCRS-40 groups than in the SCRS-0 group, however, no significant difference was observed in the plasma glucose concentration between the SCRS-20 and SCRS-40 groups (P>0.05). The plasma GluTR tended to increase (P=0.06) with increasing dietary levels of soybean curd residue silage.

The plasma acetate concentration and [1-^{13}C]acetate enrichment were also constant during the last 2 h of isotope infusion (Figures 4.5). The plasma acetate concentration was not significantly influenced by the treatments (P=0.13) (Table 4.8). The plasma AceTR tended to increase (P=0.08) with increasing dietary levels of soybean curd residue silage.

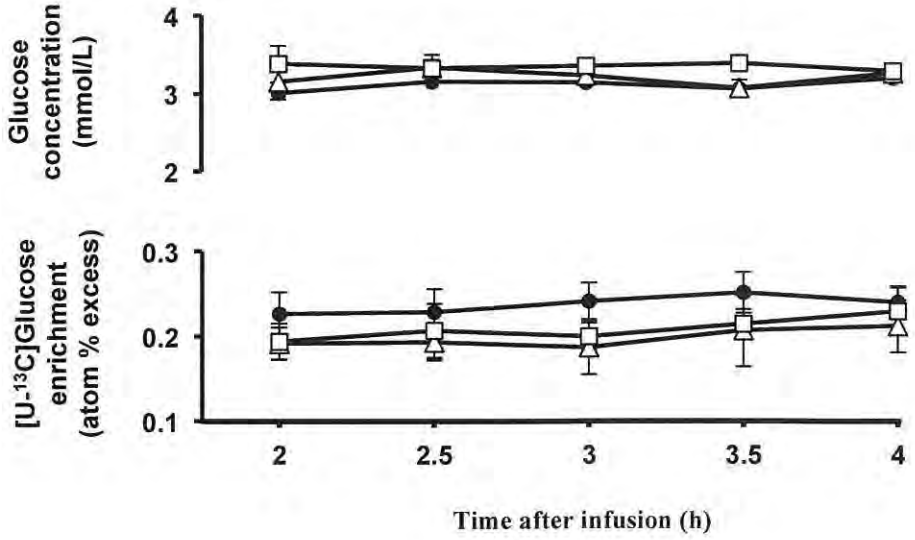


Figure 4.4. Plasma glucose concentration and enrichment of [U-^{13}C]glucose in sheep fed SCRS-0 (●), SCRS-20 (△) and SCRS-40 (□).

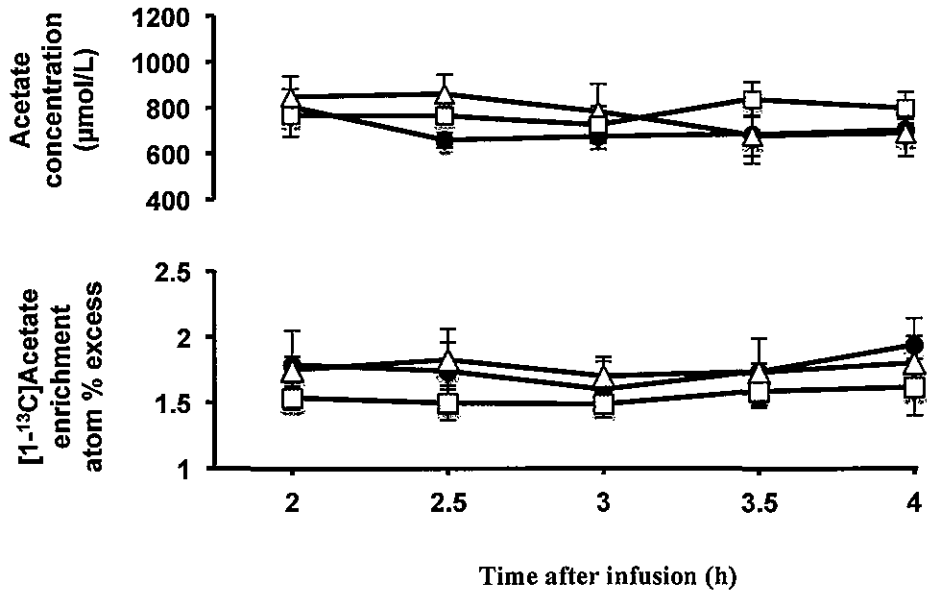


Figure 4.5. Plasma acetate concentration and enrichment of [1-^{13}C]acetate in sheep fed SCRS-0 (●), SCRS-20 (△) and SCRS-40 (□).

Table 4.8. The effects of dietary level of soybean curd residue silage on plasma glucose and acetate kinetics in sheep[1]

Item	Treatment[2]			SEM	*P* value
	SCRS-0	SCRS-20	SCRS-40		
Glucose concentration (mmol/L)	3.10[b]	3.34[a]	3.42[a]	0.10	0.03
GluTR (mmol/kg $BW^{0.75}$/h)	1.70	2.12	2.02	0.11	0.06
Acetate (μmol/L)	722	811	788	30	0.13
AceTR (mmol/kg $BW^{0.75}$/h)	5.00	5.26	6.01	0.10	0.08

[a,b,c] Means within a row with different letters differ (P<0.05)

[1] Values represent means for n = 6

[2] Treatment, partial replacement of mixed hay with soybean curd residue silage in the amount of 0% (SCRS-0, at ratio 10:0), 20% (SCRS-20, ratio at 8:2) and 40% (SCRS-40, at ratio 6:4), on DM basis.

Discussion

Silage quality

The silage was well preserved, as indicated by its low pH, high lactic acid content, high V score and Flieg point. The fermentative components were comparable with those of our previous experiments (Chapter 2 and 3).

Nitrogen metabolism

The enhancement of N intake with increasing soybean curd residue silage contents in the diet was consistent with reports in Holstein steers fed increasing amounts of soy sauce residue (Hosoda et al., 2012) and in goats fed increasing amounts of soybean meal (Kadzere and Jingura, 1993). Many studies (Sultan and Loerch, 1992; Köster et al., 1996; Castillo et al., 2001; Gleghorn et al., 2004; Sano et al., 2004; Atkinson et al., 2007) have reported that level of N intake did not affect fecal N excretion in ruminant. Interestingly, when the amount of soybean curd residue silage in the diets increased, the fecal N excretion decreased. As fecal N represents N losses through the gut that were not metabolized in the body tissue (Figure 4.6), the decrease in fecal N excretion indicating that inclusion of soybean curd residue silage in the diets increased the utilization of N. Recently, Peripolli et al. (2011) studied the intake and digestibility of forage in grazing ruminants and revealed that low fecal N excretion was correlated with high digestibility of forage. Indeed, in the present experiment, there was an increased in N digestibility with increasing amount of soybean curd residue silage in the diet.

Similarly, Mlay et al. (2003) also found lower fecal N output in heifers fed hay containing high quantity of raw soybean curd residue (420 g DM/day) compared with those fed lower quantity of soybean curd residue (210 g DM/day) due to increasing

apparent DM and organic matter digestibility in the high soybean curd residue group. Although there was tendency toward increasing N retention in the first experiment (Chapter 2), the lack of response in N retention in the present experiment may have resulted from increasing urinary N excretion.

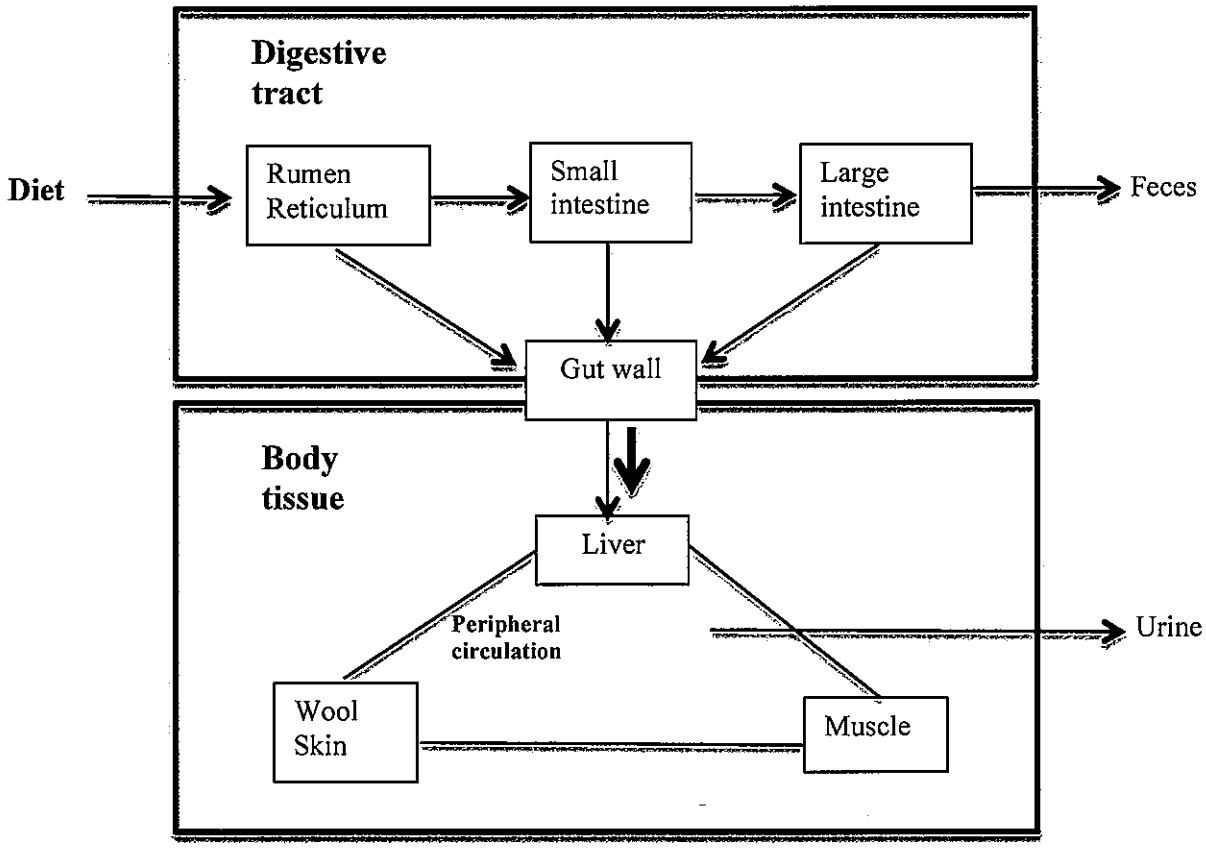


Figure 4.6. Schematic diagram illustrated the digestive tract and important body tissues of sheep that are important sites of movement and metabolism of nitrogenous compound in ruminant.

The average ruminal pH across treatments ranged from 6.4 to 6.8, which is considered suitable for fiber digestion (Djikstra, 1994). Increasing the dietary levels of soybean curd residue silage did not influence the ruminal pH, which is consistent with the results observed in dairy cows with increased dietary protein intake (Ipharraguerre

et al., 2005). The high availability of NH_3 in the rumen of sheep fed soybean curd residue silage most likely influenced the ruminal pH. The ruminal NH_3 in the SCRS-fed sheep might be rapidly absorbed into the blood, carried to the liver and converted to urea. Some of this urea might be returned to the rumen via the saliva, where it would contribute to buffering the acidity of the ruminal fluid. Indeed, in the present study, the higher concentration of NH_3 in the ruminal fluid was, the higher concentrations of NH_3 and urea in the plasma. The elevation of plasma NH_3 and urea concentrations in the present study were supported by other studies that increased the protein solubility in the diet of sheep (Wohlt et al., 1976) and increased the ruminally degradable protein supplementation in the diet of cattle (Castillo et al., 2001; Gleghorn et al., 2004).

Concerning to the protein turnover, Lobley et al. (1992) suggested that hepatic metabolism altered the amount and pattern of AAs that entered and became available in the systemic circulation, and AAs that could not be measured from the peripheral vein might be used as energy-rich substrates in the hepatic metabolism to support protein turnover within the tissues. Nonetheless, those mechanisms might be differed when soybean curd residue silage was fed. The loss of N in the SCRS-fed sheep as shown by the increased concentration of plasma urea and urinary N excretion might affect the amount of AA absorbed in the small intestine and appeared in the plasma. Although the plasma LeuTR seems to be increased slightly with increasing dietary levels of soybean curd residue silage (469, 516 and 572 $\mu mol/kgBW^{0.75}/h$ for SCRS-0, SCRS-20 and SCRS-40, respectively), these values did not attain statistical significance. In other study, Liu et al. (1995) reported that in lambs nourished with an intragastric VFA infusion containing acetate, propionate and butyrate (in molar proportions of 0.65:0.25:0.10) and incremental casein-N infusions (1500, 500 and 50 $mg/kg^{0.75}/day$), the N flux of the whole body protein turnover increased with increased levels of casein

infusion. Although in their experiment the energy supply also maintained constant, the result was contradict to our findings. Some possible causes of these differences are; first is the different types of protein used; second is the treatment, in which the casein was infused in the incremental quantities: 3, 1 and 0.1 times of N equilibrium; third is the microbial degradation of casein in the rumen might be less because casein was infused directly into the abomasum, thus influence the quantity of AA absorbed in the small intestine; and fourth is under normal condition, infant and growing animals usually have higher rate of protein turnover than those in the adulthood, as suggested by Wolfe and Chinkes (2005) and Waterlow (2006).

Furthermore, current experiment demonstrated that increasing level of dietary CP intake caused by increasing amounts of soybean curd residue silage in the diet would not enhance the protein synthesis activity of t-RNA synthetase. Previously, Taillandier et al. (1996) and Kita et al. (1996) reported that the RNA translational efficiency and fractional rate protein synthesis were depressed in rats and chicks fed a high protein (30% and 40%) diet.

Carbohydrate metabolism

As discussed in the previous chapter (Chapter 2), total VFA concentration in the rumen increased when soybean curd residue silage was fed to sheep in the amount of 20% on DM basis, but the VFA concentration did not increase further with higher amount of silage, as shown by similar concentrations between the SCRS-20 and SCRS-40 groups in the present experiment. Conversely, the highest amount of silage in the diet was, the highest concentration of NH_3 observed in the rumen. Similarly, in cattle, Yost et al. (1977) and Cunningham et al. (1996) reported that increases in the protein contents in the diet increased the concentration of NH_3 in the rumen but not the

concentration of VFA. The lack of difference in the concentration of ruminal acetate between SCRS-20 and SCRS-40 group might result from the difference in the fiber content in the diets (578 g/kg DM for SCRS-20 and 469 g/kg DM for SCRS-40), as the acetate concentration in the rumen is positively correlated with the dietary fiber (Bergman et al., 1990). In other hand, increasing levels of soybean curd residue silage in the diets seems to increase the availability of starch. In addition to starch, lactic acid in soybean curd residue silage also converted to propionate in the rumen, as suggested by Gill et al. (1986). Although ruminal propionate concentration in the SCRS-40 (15.2 mmol/L) seems to be higher than those in the SCRS-20 (14.3 mmol/L), the values did not attain statistical significance. Moreover, the presence of lactic acid in the diet from silage is likely associated with the elevated concentration of plasma lactate in the SCRS-20 and SCRS-40 groups. Similar results were observed in sheep fed rye grass silage (Gill et al., 1986) and in dairy cows fed barley silage (Ametaj et al., 2009). In ruminants, plasma NEFA is considered to be a good nutritional status indicator (Bowden et al., 1971) because NEFA is released into the blood when fat is mobilized to supply the metabolic needs of the animal, primarily when the other available energy is not sufficient. Hence, the comparable plasma NEFA concentrations across treatments indicated the positive effect of soybean curd residue silage on the energy supply in sheep.

The contribution of propionate to glucose synthesis has been studied extensively, as discussed in Chapter 2 and 3. Several studies suggested that when the ME intakes were kept isoenergetic, the GluTR was similar for either roughage or concentrate based-diets in cattle (Herbein et al., 1978; Harmon et al., 1983; Schmidt and Keith, 1983), lambs (Kempton and Leng, 1983) and sheep (Judson et al., 1968; Ulyatt et al., 1970). Although in the current experiment the ME intakes also kept isoenergetic, the

plasma GluTR tended to increase with increasing amount of soybean curd residue silage in the diet. Nonetheless, when the SCRS-40 diet was fed to sheep, the plasma GluTR was similar with those in the SCRS-20. The comparable results between the SCRS-20 and SCRS-40 are likely associated with similar concentrations of ruminal propionate in the two diets, indicating that high carbohydrate availability in the diets did not further increase both ruminal propionate concentration and plasma GluTR in sheep.

The plasma AceTR were comparable to those observed in sheep fed mixed clover and alfalfa hay measured using [1-^{14}C]acetate as a tracer (Sabine and Johnson, 1964), as well as those in sheep fed mixed orchardgrass and reed canarygrass hay measured using [1-^{13}C]acetate as a tracer (Alam et al., 2010). Previous studies on whole body acetate turnover in cattle and sheep reported that acetate utilization did not depend on the acetate concentration (Lee and Williams, 1962; Al-Mamun et al., 2009). Our findings are consistent with these reports. In other study, Prior et al. (1976) reported that level of intake did not significantly influence plasma AceTR, as shown by similar plasma AceTR between sheep fed at maintenance and those fed at twice of maintenance level, measured with [^{14}C]acetate. In current study, although plasma AceTR levels among treatments also did not attain statistical significance, there was a slight increase in the AceTR with increasing level of soybean curd residue silage supplementation. This trend was probably influenced by increasing amount of soluble carbohydrate in the diets, as suggested by Ballard et al. (1972). The NFC contents in the diets were increased with increasing level of soybean curd residue silage supplementation; 70, 162 and 254 g/kg DM for SCRS-0, SCRS-20 and SCRS-40, respectively.

Conclusion

The increasing dietary levels of soybean curd residue silage resulted in increased ruminal NH_3 concentration, but total ruminal VFA concentration was less affected. Although urinary N excretion was elevated with increasing amount of the silage, plasma LeuTR did not affected. Moreover, current study indicated that increasing dietary levels of soybean curd residue silage did not significantly influence plasma Leu, glucose and acetate kinetics if the diets were isoenergetic.

Chapter 5

General Discussion

Thesis outline

The focus of this thesis was to evaluate the effects of feeding soybean curd residue silage to sheep. This by-product is considered as a promising potential feed for ruminants because of high contents of protein and carbohydrate. Previous studies revealed that soybean curd residue is palatable to ruminants, and dietary supplementation with this product increased milk production as well as the milk protein, milk lactose and milk fat contents in dairy cows (Chiou et al., 1998). The raw and ensilaged forms of soybean curd residue were reported to have effects comparable to those of commercial concentrates on fattening sheep (Kakihara et al., 2004) and increasing body weight gain and lipid composition in goats (Hirayama et al., 2002). The main research questions were how soybean curd residue silage supplementation affects the protein, glucose and fatty acid metabolism in the animal body.

Crossbred (Suffolk × Corriedale) sheep were subjected to various feeding treatments in these studies. The effects of inclusion of soybean curd residue silage in a roughage diet (Chapter 2) demonstrated that this by-product could be used as an alternative protein and carbohydrate sources for sheep without any detrimental effects on plasma Leu or glucose kinetics. After briefly discussing the feeding effects of soybean curd residue silage, the goal of the next section (Chapter 3) was to compare the nutrient metabolism of two different feed; soybean curd residue silage and commercial concentrate. It was previously speculated that protein and glucose metabolism and their regulation would not be affected by replacing the commercial concentrate in the diet with soybean curd residue silage. After comparable results between soybean curd residue silage and commercial concentrates were obtained, the next chapter (Chapter 4) examined the effects of different levels of soybean curd residue silage on the whole

body nutrient metabolism in sheep. The effects on plasma acetate kinetics were investigated, in addition to the plasma Leu and glucose kinetics as studied in the previous sections. Together, these studies described the effects of feeding different amounts of soybean curd residue silage on whole body protein, glucose and acetate metabolism in sheep. Finally, the current work to obtain insight into the physiological and nutritional effects of food residues in the ruminant diet is outlined, and the major conclusions of the thesis are summarized.

Utilization of nitrogenous compounds

Some feed protein can escape from rumen degradation and another are degraded by microorganisms in the rumen, which produce NH_3, VFA, CO_2 and other metabolites. However, there are outstanding questions as how the nutrients in food by products affect the metabolism in animal body and the value of it.

In the soybean curd manufacturing process, soybeans are boiled before grinding and filtering. These processes should have profound effects on the solubility of the resulting residue. Furthermore, during the ensiling process, extensive protein hydrolysis occurs, which results in more soluble N fraction. Soluble N is generally considered to be easily degraded by microbes in the rumen and to affect the digestibility in host animals (McDonald et al., 2011). Many reports have confirmed that supplementation with soybean curd residue (Chiou et al., 1998; Mlay et al., 2003; Cao et al., 2009; Hosoda et al., 2012) in the roughage diets increased N intake and digestibility. Increasing the N content of the diet through supplementation of soybean curd residue silage also increased the rumen concentration of NH_3, thereby potentially improving both microbial growth and cellulolytic activity in the rumen. As most typical roughage

contained a large fraction of protein bound to the cell wall (Aufrère and Guérin, 1996), improved cellulolysis may have led to increase accessibility of the protease enzymes responsible for protein degradation, thereby increasing protein degradability. This may explain the reason why fecal N excretion was lower when soybean curd residue silage was fed to sheep (Chapter 2), and when the amount of soybean curd residue silage in the diet was increased, the fecal N excretion was decreased significantly (Chapter 4).

McDonald et al. (2011) stated that highly soluble protein generally promoted lower N retention than did poorly soluble protein. This statement contradicts the current findings because there was tendency toward increasing N retention in sheep fed soluble protein from soybean curd residue silage (Chapter 2), and compared with commercial concentrate, the amount of N retained in sheep were similar (Chapter 3). Nonetheless, when the dietary level of silage increased (Chapter 4), the N retention was not significantly affected. Elevated urinary N excretion as a consequence of excess protein may have influenced the pattern of N retention in Chapter 4.

In this study, the concentrations of ruminal NH_3 were 6.6 and 7.9 mmol/L in sheep fed hay alone; 10.9, 11.3 and 11.9 mmol/L in sheep fed 80% hay plus 20% soybean; and 12.9 mmol/L in sheep fed 60% hay plus 40% soybean curd residue. It appears that soybean curd residue silage enhanced the concentration of ruminal NH_3. Moreover, all ruminal NH_3 concentrations in the SCRS-fed sheep were in the range that had been suggested preferable for microbial growth to support microbial protein synthesis (10-20 mmol/L, Song and Kennelly, 1989; Russel et al., 1992). With respect to ruminal degradation, Given and Rulquin (2004) suggested that although the degradation of silage protein in the rumen is rapid and extensive, up to 10% of silage protein could escape the rumen and absorbed in the small intestine. Nonetheless, even

when the absolute amount of AAs supplied was optimized by soybean curd residue silage supplementation, there is still catabolism (oxidation) of AA by the sheep tissues with a consequent loss of the AA that could otherwise be used to synthesize protein, as suggested by Lobley (2003).

There are several possible reasons why the dietary N intake and ruminal NH_3 concentration increased but the plasma AA concentration was not affected by soybean curd residue silage supplementation. First, the proteolytic activity during the fermentation process could alter the composition of free AAs in the silage (Ohshima and McDonald, 1978); second, AAs from a soybean-based diet may be rapidly catabolized in the body, as suggested by Deutz et al. (1998) and Bos et al. (2003); and third, a large amount of NH_3 was absorbed from the rumen into the circulation, converted into urea in the liver and excreted via urine, as illustrated in Figure 5.1. Although some N might be recycled back to the rumen as urea from blood and with saliva flow, Djikstra et al. (1992) suggested that the extent of capture is generally limited due to a lack of energy. It is also assumed that a high NH_3 concentration in the ruminal fluid depressed the transport of urea from blood to the rumen (Baldwin et al., 1987) and that the recycled N is absorbed again as NH_3 when not rapidly incorporated in microbial mass (Clark et al., 1992).

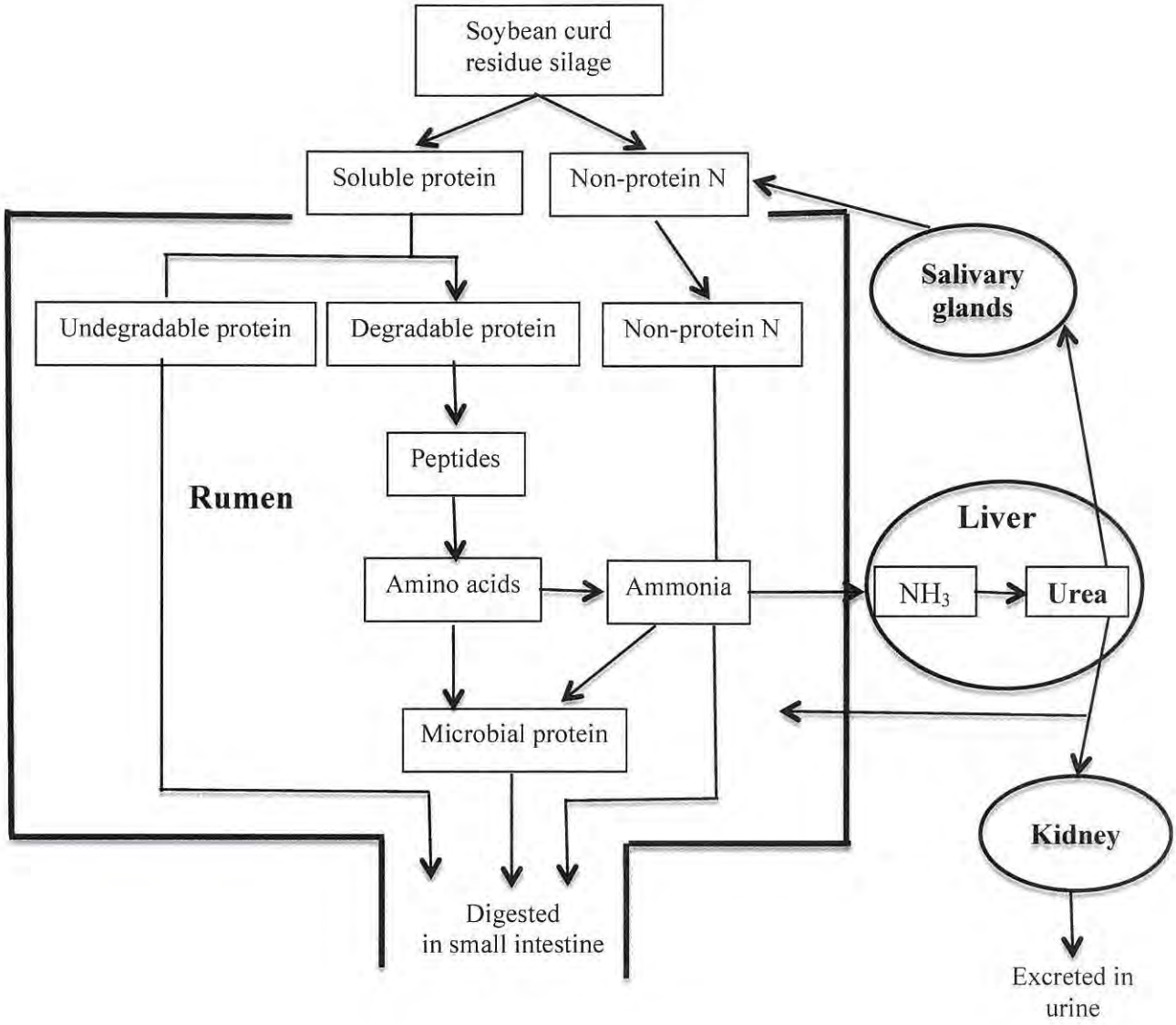


Figure 5.1. Digestion and metabolism of nitrogenous compounds from soybean curd residue silage. Degradable protein and NPN compounds in the soybean curd residue silage are converted to NH_3 in the rumen. Some of the protein can escape degradation in the rumen and subsequently digested in the small intestine. The ruminal NH_3 produced together with some small peptides and free AAs, are utilized by ruminal microbes to synthesize the microbial protein. On other hand, when NH_3 is accumulated in the rumen, the concentration will be exceeded. The NH_3 is then absorbed into the blood, carried to the liver and converted to urea. Some of this urea may be returned to the rumen via the saliva, but most is excreted in the urine.

Lobley et al. (1987) studied the effect of a progressive reduction in feed intake on WBPS using the [1-^{14}C]Leu as tracer in finishing beef steers and found that feed

intake influenced overall protein metabolism. Hence, it was previously expected that increasing dietary N intake through soybean curd residue silage feeding influenced the protein turnover and WBPS. The plasma LeuTR levels were 446 and 469 μmol/kgBW$^{0.75}$/h in sheep fed only hay; 507, 514 and 516 μmol/kgBW$^{0.75}$/h in sheep fed 80% hay plus 20% soybean curd residue; and 572 μmol/kgBW$^{0.75}$/h in sheep fed 60% hay plus 40% soybean curd residue. Although the plasma LeuTR seems to be slightly increased when soybean curd residue silage was fed, but these values were not significantly different. From the present study, it appears that, when the diets are isoenergetic or when the protein supplies are above the animal requirement, whole body protein turnover and protein synthesis are less affected. With different levels of energy intake, previous studies reported an increase in WBPS with increasing ME intake in sheep (Harris et al., 1992; Savary et al., 2001), goats (Fujita et al., 2007) and steers (Lapierre et al., 1999). Moreover, the aminoacyl-tRNA synthetase activity and mRNA availability would not be enhanced with high CP intake (Kita et al., 1996; Taillandier et al., 1996). Overall, the present findings showed that the commercial concentrate and soybean curd residue silage produce similar responses in plasma Leu kinetics in diets formulated with the same energy intake.

Volatile fatty acid and glucose metabolism

Soybean curd residue silage is rich in fermentable carbohydrates, which are broken down in the rumen to acetate, propionate and butyrate and small amounts of branched-chain fatty acids, as illustrated in Figure 5.2. Bergman (1990) suggested that VFA could contribute up to 70% of the caloric requirements of ruminants. In sheep, the total ruminal VFA could be increased by incorporating soybean curd residue silage into

the diet. Moreover, the concentration of total ruminal VFA resulting from soybean curd residue silage supplementation was comparable with that obtained with commercial concentrate supplementation in isonitrogenous and isoenergetic diets.

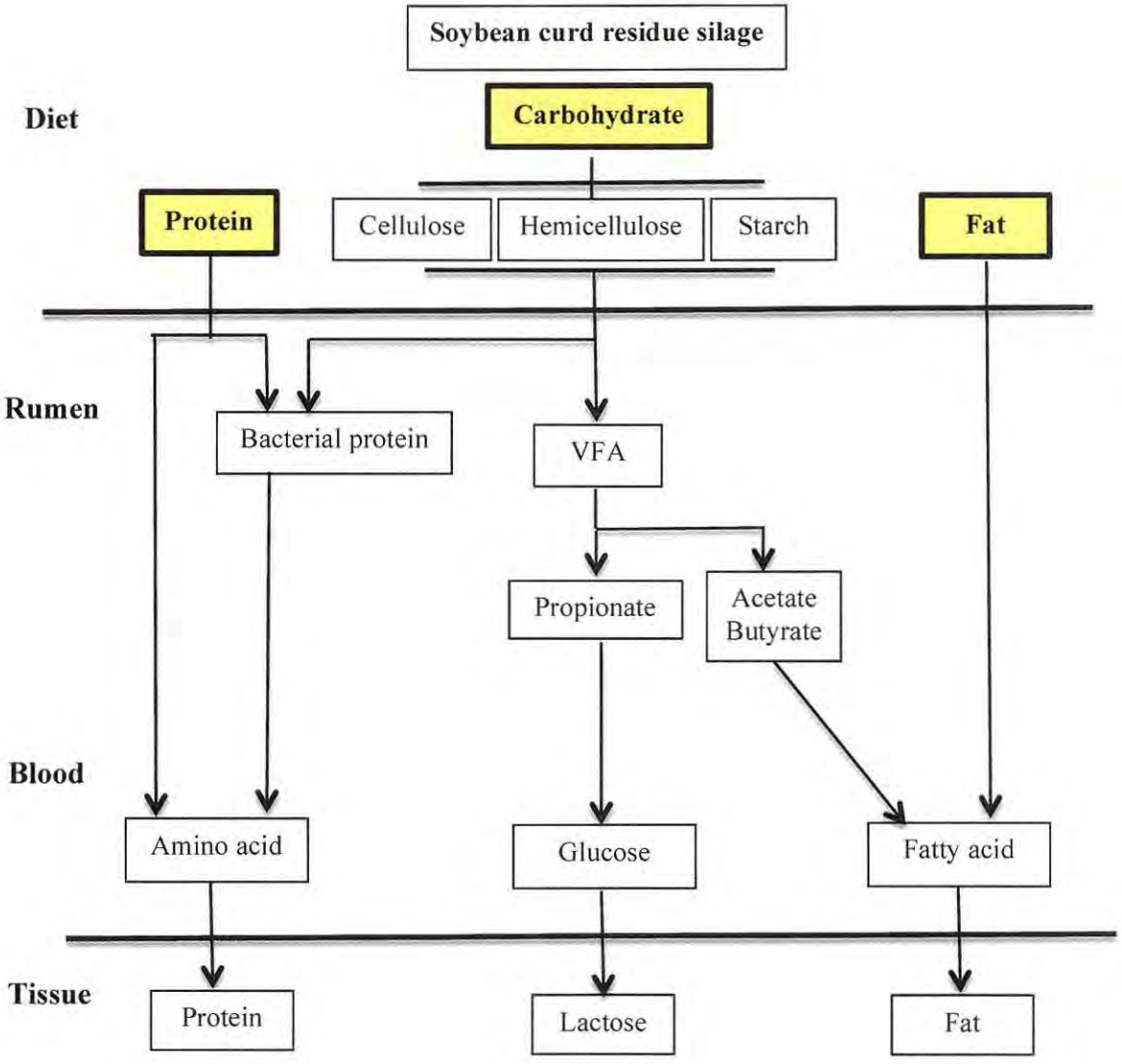


Figure 5.2. Digestion and metabolism of carbohydrate compounds. Carbohydrates in soybean curd residue silage are fermented to VFAs (mainly acetate, propionate and butyrate) by microorganisms in the rumen and used as energy to synthesize microbial protein. Propionate is considered a substrate for gluconeogenesis, whereas acetate and butyrate are utilized as substrates for lipogenesis. Adapted from Church (1979).

The present study showed that the concentration changes in plasma acetate and propionate were reflected by corresponding changes in the concentrations of these substances in the rumen. It has also have been demonstrated that soybean curd residue silage could enhance the availability of acetate in the animal body. Acetate is used as an energy source for ruminants (Djikstra et al., 2005). Moreover, some metabolic studies with ^{14}C-labelled acetate revealed that acetate is the major precursor of lipogenesis in the ruminant tissue (Hanson and Ballard, 1967; Bauman et al., 1972; Ingle et al., 1972; Greathead et al., 2001). Indeed, Hirayama et al. (2002) reported an increase in lipid concentration in the carcasses of goats fed wild grass supplemented with soybean curd residue (at a ratio of 7:3 DM). In another fattening study, Kakihara et al. (2004) reported that the lipid composition in the carcasses of lambs fed soybean curd residue was comparable to that in lambs fed a commercial concentrate. Hence, the present findings indicated that soybean curd residue silage also has a positive impact on intermediary acetate metabolism in sheep.

The fermentable carbohydrate content in soybean curd residue silage, which is mainly starch, increased the ruminal propionate concentration in sheep. Seal et al. (1992) suggested that 53% of the whole body glucose is derived from propionate. In the three different experiments in the present study, the plasma GluTR levels were 1.70 and 1.76 mmol/kgBW$^{0.75}$/h in sheep fed only hay; 2.10, 2.12 and 2.14 mmol/kgBW$^{0.75}$/h in sheep fed 80% hay plus 20% soybean curd residue silage; and 2.02 mmol/kgBW$^{0.75}$/h in sheep fed 60% hay plus 40% soybean curd residue silage. Although the plasma GluTR level seems to increase with soybean curd residue silage supplementation, this difference did not attain statistical significance. Moreover, the plasma GluTR measured in sheep fed hay supplemented with soybean curd residue silage was comparable to

those measured in sheep fed hay supplemented with concentrate (2.24 mmol/kgBW$^{0.75}$/h). Taken together, current findings demonstrated that comparable plasma GluTR can be obtained among dietary treatments as long as the diets are kept isoenergetic.

Chapter 6

Summary and General Conclusion

Summary

The use of residues generated from the food industry as livestock feed has increased throughout the world as the result of increasing demand for livestock products and increasing feed prices. The selection of safe feed material is important because animal protein cannot be used in ruminant feed. Consequently, application of industrial residues containing plant proteins has become a matter of interest for many ruminant nutritionists. The use of these residues will provide additional income for the food industry and reduce environmental problems related to residue disposal. For the farmer, the use of food residues, which are generally less expensive than other commercial feeds, will reduce feed costs. Soybean curd residue is a promising potential alternative feed due to its high protein and carbohydrate contents. The ensiling process not only lengthens the useful life of the soybean curd residue but also eliminates many of its anti-nutritional properties and increases its digestibility in animals. Therefore, three different experiments were conducted to investigate the feeding effects of soybean curd residue silage on whole body nutrient metabolism in ruminant.

In the first experiment, the effects of inclusion of soybean curd residue silage in roughage diet on the plasma leucine (Leu) and glucose kinetics in sheep were assessed by an isotope dilution method of [1-^{13}C]Leu and [U-^{13}C]glucose. Two different dietary treatments were tested, one was mixed orchardgrass and reed canarygrass hay (Hay diet) and another one (SCRS diet) was mixed hay plus soybean curd residue at a ratio of 8:2 on a dry matter (DM) basis. The metabolizable energy (ME) intakes were formulated slightly above the maintenance level for both diets. The experiment followed a crossover design with a period of 21 days. Primed-continuous infusion of [1-^{13}C]Leu and [U-^{13}C]glucose was performed on day 21. The nitrogen (N) intake, N absorption and N digestibility were enhanced by the SCRS diet (P<0.0001, P<0.0001

and P=0.001, respectively). Although the urinary N excretion was elevated (P=0.008), the fecal N excretion was low (P=0.001), producing an overall tendency (P=0.07) toward high N retention in the SCRS-fed sheep. The concentrations of ruminal ammonia (NH_3) and total volatile fatty acid (VFA) were increased (P=0.01 and P=0.03) when soybean curd residue silage was fed to sheep. The total VFA concentration in the blood tended to be higher (P=0.05) for the SCRS-fed sheep than those fed hay alone. The plasma Leu turnover rate (LeuTR), whole body protein synthesis and degradation (WBPS and WBPD) remained similar between the treatments. The plasma glucose concentration tended to be higher (P=0.06) for sheep fed the SCRS diet than those fed the Hay diet, whereas the plasma glucose turnover rate (GluTR) did not differ between the two groups. These findings suggest the feasibility of soybean curd residue silage as protein and carbohydrate sources for sheep.

In the second experiment, the effects of replacing commercial concentrate with soybean curd residue silage on the plasma Leu and glucose kinetics in sheep were assessed by an isotope dilution method of [1-^{13}C]Leu and [U-^{13}C]glucose. Two different dietary treatments, one consisted of 80% mixed hay and 20% commercial concentrate (CONC diet), and another one (SCRS diet) consisted of 80% mixed hay and 20% soybean curd residue silage were tested. The dietary treatments were estimated to be isonitrogenous, and the ME intakes were slightly above the maintenance level for both diets. The experiment followed a crossover design with a period of 21 days. Primed-continuous infusion of [1-^{13}C]Leu and [U-^{13}C]glucose was performed on day 21. Sheep fed the SCRS diet absorbed and retained similar amounts of N compared with sheep fed the CONC diet. Moreover, the N digestibility was comparable between the two diets. The plasma LeuTR was comparable between the SCRS and CONC diets, and no differences were noted in the WBPS and WBPD between the two diets. The

concentrations of ruminal NH_3 and total VFA were similar for both diets. The propionate concentration in the rumen was higher (P=0.02) for the SCRS diet than for the CONC diet, whereas acetate concentrations were similar between the diets. The plasma glucose concentrations and plasma GluTR were also comparable between the diets. These findings showed that feeding soybean curd residue silage resulted in similar plasma Leu and glucose kinetics with those produced from feeding commercial concentrate.

In the third experiment, the effects of dietary level of soybean curd residue silage on the plasma Leu, glucose and acetate kinetics in sheep were studied using an isotope dilution method of [1-^{13}C]Leu, [U-^{13}C]glucose and [1-^{13}C]Na acetate simultaneously. Sheep were fed three diets differing in the amount of soybean curd residue silage: 100% mixed hay (SCRS-0, as a control), 80% mixed hay plus 20% soybean curd residue silage (SCRS-20), and 60% mixed hay plus 40% soybean curd residue silage (SCRS-40) on a DM basis. The ME intakes were formulated slightly above the maintenance level for all diets. The experiment followed a 3×3 Latin square design for 21 days. The N intake and N digestibility were increased with increasing amounts of soybean curd residue silage in the diets (P<0.0001 and P=0.001). Increased dietary level of soybean curd residue silage decreased (P<0.001) the fecal N excretion but increased (P<0.001) the urinary N excretion. The N retention, plasma LeuTR and WBPS did not differ among the treatments. The ruminal pH did not differ among the treatments. The ruminal NH_3 concentration increased (P=0.001) with increasing amount of soybean curd residue silage in the diets. Nonetheless, high level of feeding did not further increase the concentrations of ruminal total VFA, acetate and propionate, as indicated by similar concentrations of those acids in the SCRS-20 and SCRS-40 groups. Moreover, this study showed that the plasma GluTR and acetate turnover rate (AceTR)

tended to increase (P=0.06 and P=0.08) with increasing amount of silage in the diets. These findings suggested that the dietary level did not significantly influence the plasma Leu, glucose and acetate kinetics when the diets were kept isoenergetic.

General conclusion

Taken together, the results of the present study demonstrated that soybean curd residue silage should not be considered as a waste product; rather, it is a potential energy resource. It has been proved that soybean curd residue silage did not adversely affect the protein, glucose and acetate metabolism due to its high soluble N and fermentable carbohydrate contents. The use of soybean curd residue as a feed represents a better way of recycling food industry wastes back to human nutrition through animal feeding and the present findings will contribute to the sustainable ruminant nutrition.

ヒツジにおける全身の栄養素代謝に及ぼす豆腐粕給与の影響に関する研究

畜産物需要の増加および飼料価格の高騰の結果、食品産業から生じる食品残渣の飼料としての利用が世界的に増加している。反芻家畜の飼料に動物性タンパク質は使用できないので、安全な飼料原料の選択が重要である。その結果、植物性タンパク質を含む産業廃棄物の応用が反芻家畜栄養学に携わる研究者の課題になっている。これらの残渣の使用は食品産業の副収入となり、残渣廃棄に関連する環境問題を軽減することができる。畜産農家にとっては、一般的に他の市販飼料よりも安価な食品残渣の利用は飼料費を軽減することができる。豆腐粕はタンパク質や炭水化物を豊富に含んでいるため、有望な代替飼料になり得る。さらに、サイレージ調製は、豆腐粕の使用期間を延長することができるばかりでなく、多くの抗栄養成分を消失させ、飼料消化率を増加させることができる。したがって、反芻家畜における全身の栄養素代謝に及ぼす豆腐粕サイレージ給与の影響を測定するため、３つの実験を実施した。

実験１では、ヒツジの血漿ロイシン(Leu)およびグルコース代謝に及ぼす粗飼料への豆腐粕サイレージ添加の影響を[1-^{13}C]Leu および[U-^{13}C]グルコースの同位元素希釈法を用いて測定した。飼料区はオーチャードグラスとリードカナリーグラスの混播乾草(MH 区)および混播乾草＋豆腐粕サイレージ(8:2 乾物、SCRS 区)とした。代謝エネルギー(ME)給与量を等量とし、2 区とも維持量をわずかに上回る量とした。実験期間は 1 期 21 日とし、クロスオーバー法にしたがって実施した。21 日目に[1-^{13}C]Leu および[U-^{13}C]グルコースの primed-continuous infusion 法を実施した。窒素(N)摂取量、N吸収量、N消化率は SCRS 区が MH 区より高かった($P < 0.0001$、$P < 0.0001$、$P = 0.001$)。SCRS 区において尿中N排泄量は高かったが($P = 0.008$)、糞中N排泄量は低く($P = 0.001$)、全体としてN蓄積は高い傾向を示した($P = 0.07$)。ルーメン内アンモニア(NH_3)および総揮発性脂肪酸(VFA)濃度は豆腐粕サイレージ添加によって増加した($P = 0.01$、

P = 0.03)。血液 VFA 濃度は豆腐粕サイレージ添加によって増加する傾向を示した(P = 0.05)。血漿 Leu 代謝回転速度(LeuTR)、全身のタンパク質合成速度(WBPS)および分解速度(WBPD)は飼料区間で類似していた。血漿グルコース濃度は豆腐粕サイレージ添加によって増加する傾向を示したが(P = 0.06)、血漿グルコース代謝回転速度(GluTR)に有意な変化は観察されなかった。これらの知見はヒツジにとってタンパク質、炭水化物源としての豆腐粕サイレージの可能性を示している。

実験 2 では、ヒツジの血漿 Leu およびグルコース代謝に及ぼす濃厚飼料の豆腐粕サイレージへの代替への影響を[1-^{13}C]Leu および[U-^{13}C]グルコースの同位元素希釈法を用いて測定した。飼料区は混播乾草 80%＋20%市販濃厚飼料(CONC 区)および混播乾草 80%＋20%豆腐粕サイレージ(SCRS 区)とした。両飼料区ともN給与量を等量とし、ME 給与量は維持量をわずかに上回る程度とした。実験期間は 1 期 21 日とし、クロスオーバー法にしたがって実施した。21 日目に[1-^{13}C]Leu および[U-^{13}C]グルコースの primed-continuous infusion 法を実施した。SCRS 区のN吸収量、N保持量、N消化率は CONC 区と類似していた。血漿 LeuTR は SCRS 区と CONC 区とで類似しており、WBPS および WBPD は飼料区間に差がなかった。ルーメン内 NH_3 および VFA 濃度は両飼料区で類似していた。プロピオン酸濃度は CON 区より SCRS 区が高かったが(P = 0.02)、酢酸濃度は両飼料区で類似していた。血漿グルコース濃度および GluTR もまた両飼料区で類似していた。これらの知見は豆腐粕サイレージ添加給与時の血漿 Leu およびグルコース代謝は濃厚飼料給与時と類似していることを示している。

実験 3 では、ヒツジの血漿 Leu、グルコースおよび酢酸代謝に及ぼす豆腐粕サイレージ給与水準の影響を[1-^{13}C]Leu、[U-^{13}C]グルコースおよび[1-^{13}C]酢酸の同位元素希釈法を用いて測定した。3 飼料区で実験が行われた。飼料区は混播乾草の 0%、20%、40%(乾物)を豆腐粕サイレージで代替し、それぞれ SCRS-0 区、SCRS-20 区、SCRS-40 区とした。いずれの飼料区とも ME 給与量を等量とし、維持量をわずかに上回る程度

とした。実験期間は1期 21 日とし、3x3ラテン方格法にしたがって実施した。21 日目に[1-^{13}C]Leu、[U-^{13}C]グルコースおよび[1-^{13}C]酢酸 Na の primed-continuous infusion 法を実施した。N摂取量およびN消化率は豆腐粕サイレージ給与水準が増加するにしたがい増加した($P < 0.0001$、$P = 0.001$)。豆腐粕サイレージ給与水準の増加は、糞中 N 排泄量を減少させ($P < 0.001$)、尿中N排泄量を増加させた($P < 0.001$)。N保持量、血漿 LeuTR および WBPS は飼料間に差がなかった。豆腐粕サイレージ給与水準の増加はルーメン内 NH_3 濃度を増加させたが($P = 0.001$)、ルーメン pH に悪影響を及ぼさなかった。ルーメン内酢酸およびプロピオン酸濃度は豆腐粕サイレージ添加によって増加したが($P < 0.05$)、SCRS-20 区と SCRS-40 区との間には差がなかった。血漿グルコース濃度は豆腐粕サイレージ給与水準の増加によって増加した(P=0.03)。さらに、血漿 GluTR および酢酸代謝回転速度(AceTR)は豆腐粕サイレージ給与水準が増加するにしたがい増加する傾向を示した($P = 0.06$、$P = 0.08$)。以上の結果から、添加水準はエネルギー給与量が一定の場合、豆腐粕サイレージ給与水準が血漿 Leu、グルコースおよび酢酸代謝に有意な影響を与えないことが示された。

本研究で得られた結果をまとめると、豆腐粕は廃棄物ではなく、有望なエネルギー資源であることが証明された。豆腐粕サイレージ中の高可溶性Nおよび易発酵性炭水化物はヒツジのタンパク質、グルコースおよび酢酸代謝に悪影響を及ぼさないことが示された。飼料としての豆腐粕の使用は、家畜飼養を通じて食品加工残渣を人の栄養に再利用できる優れた方法であり、本研究の知見は持続可能な反芻家畜栄養に貢献できるであろう。

References

Alam, M.K., Ogata, Y., Sako, K., Al-Mamun, M., Sano, H., 2010. Intermediary metabolism of plasma acetic acid, glucose and protein in sheep fed rice straw-based diet. Asian-Aust. J. Anim. Sci. 23, 1333-1339.

Allen, M.S., Bradford, B.J., Oba, M., 2009. Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87, 3317-3334.

Al-Mamun, M., Goto, K., Chiba, S., Sano, H., 2009. Response of plasma acetate metabolism to Hop (*Humulus lupulus L.*) in sheep. Int. J. Biol. Sci. 5, 287-292.

Amaha, K., Sasaki, Y., Segawa, T., 1996. Utilization of tofu (soybean curd) by-product as feed for cattle. ASPAC Food and Feed Fertilizer Centre. http://www.agnet.org/library/eb/419[1]pdf. Accessed May 14, 2012.

Ametaj, B.N., Emmanuel, D.G.V., Zebeli, Q., Dunn, S.M., 2009. Feeding high proportions of barley grain in a total mixed ration pertubs diurnal patterns of plasma metabolites in lactating dairy cows. J. Dairy Sci. 92, 1084-1091.

Annison, E.F., Hill, K.J., Lewis, D., 1957. Studies on the portal blood of sheep: Absorption of volatile fatty acids from the rumen of the sheep. J. Biochem. 66, 593-599.

AOAC., 1990. Official Methods of Analysis.15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Atkinson, R., Toone, C.D., Robinson, T.J., Harmon, D.L., Ludden, P.A., 2007. Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on nitrogen retention, apparent digestibility, and nutrient flux across visceral tissues in lambs fed low-quality forage. J. Anim. Sci. 85, 3331-3339.

Aufrère, J., Guérin, H., 1996. Critical review of chemical and enzymatic methods for the estimation of nutritive value in roughages. Ann. Zootech. 45, 21-38.

Baldwin, R.L., Thornley, J.H.M., Beever, D.E., 1987. Metabolism of lactating cow. II. Digestive elements of a mechanistic model. J. Dairy Res. 54, 107-131.

Ballard, F.J., Filsell, O.H., Jarrett, I.G., 1972. Effects of carbohydrate availability on lipogenesis in sheep. Biochem. J. 226, 193-200.

Bauman, D.E., Dekay, D.E., Ingle, D.L., Brown, R.E., 1972. Effect of glycerol and glucose additions on lipogenesis from acetate in rat and cow mammary tissue. Comp. Biochem. Physiol. 43, 479-486.

Beauchemin, K.A., 1991. Effects of dietary neutral detergent fiber concentration and alfafa hay quality on chewing, rumen function, and milk production of dairy cows. J. Dairy Sci. 74, 3140-3151.

Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70, 567-590.

Bos, C., Metges, C.C., Gaudichon, C., Petzke, K.J., Pueyo, M.E., Morens, C., Everwand, J., Benamouzig, R., Tome, D., 2003. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J. Nutr. 133, 1308-1315.

Bowden, D.M., 1971. Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: A review. Can. J. Anim. Sci. 51, 1-13.

Bradford, G.E., 1999. Contribution of animal agriculture to meeting global human food demand. Livest. Prod. Sci. 59, 95-112.

Calder, A.G., Smith, A., 1988. Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry.

Use of tertiary butyldimenthylsilyl derivatives. Rapid Comm. Mass Spectrom. 2, 4-16.

Cao, Y., Takahashi, T., Horiguchi, K., 2009. Effects of addition of food-by products on the fermentation quality of a total mixed ration with whole crop rice and its digestibility, preference, and rumen fermentation in sheep. Anim. Feed Sci. Technol. 151, 1-11.

Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C., France, J., 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci. 79, 247-253.

Chiou, P.W.S., Chen, C.R., Chen, K.J., Yu, B., 1998. Wet brewers' grains or bean curd pomace as partial replacement of soybean meal for lactating cows. Anim. Feed Sci. Technol. 74, 23-134.

Cho, J.H., Min B.J., Chen, Y.J., Yoo, J.S., Wang, Q., Kim, J.D., Kim, I.H., 2007. Evaluation of FSP (Fermented Soy Protein) to replace soybean meal in weaned pigs: growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. Asian-Aust. J. Anim. Sci. 20, 1874-1879.

Church, D.C., 1979. Digestive Physiology and Nutrition of ruminants. Vol 1. Digestive Physiology. 2nd Ed. Corvallis, O and B Books.

Clark, J.H., Klusmeyer, T.H., Cameron, M.R., 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of Dairy Cows. J. Dairy Sci. 75, 2304-2323.

Clifford, A.J., Tillman, A.D., 1968. Urea and isolated soybean protein in sheep purified diets. J. Anim. Sci. 27, 484-489.

Cunningham, K.D., Cecava, M.J., Johnson, T.R., Ludden, P.A., 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79, 620-630.

Deutz, N.E.P., Bruins, M.J., Soeters, P.B., 1998. Infusion of soy and casein protein meals affects interorgan amino acid metabolism and urea kinetics differentially in pigs. J. Nutr. 128, 2435-2445.

Dhaouadi, A., Monser, L., Sadok, S., Adhoum, N., 2007. Validation of flow-injection-gas diffusion method for total volatile basic nitrogen determination in seafood products. J. Food Chem. 103, 1049-1053.

Djikstra, J., 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39, 61-69.

Djikstra, J., Forbes, J.M., France, J., 2005. Quantitative aspects of ruminant digestion, 2nd Ed., CABI publishing, UK.

Djikstra, J., Neal, H.D.St.C., Beever, D.E., France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen in the rumen: model description. J. Nutr. 122, 2239-2256.

Enishi O., Masaki, S., Saeki, M., Kawashima, T., Nonaka, I., Nakashima, K., 2004. Evaluation of feedstuffs using some physicochemical parameters. Grassland Sci. 50, 399-407.

Evans, E., Buchanan-Smith, J.G., MacLeod, G.K., 1975. Postprandial patterns of plasma glucose, insulin and volatile fatty acids in ruminants fed low- and high-roughage diets. J. Anim. Sci. 41, 1474-1479.

FDA (U.S. Food and Drug Administration)., 2004. CVM and ruminant feed (BSE) inspections. Rule 21 CFR, part 589.2000. Code of Federal Regulations. Center for

Veterinary Medicine, FDA, Washington,http://www.fda.gov/cvm/RuminantFeed Inspections.htm. Accessed March 12, 2012.

Foster, J.L., Adesogan, A.T., Carter, J.N., Blount, A.R., Myer, R.O., Phatak, S.C., 2009. Intake, digestibility, and nitrogen retention by sheep supplemented with warm-season legume hays or soybean meal. J. Anim. Sci. 87, 2891-2898.

Fujita, T., Kajita, M., Sano, H., 2007. Effects of non-protein energy intake on whole body protein synthesis, nitrogen retention and glucose turnover in goats. Asian Aust. J. Anim. Sci. 20, 536-542.

Fujita, T., Majima, H., Itoh, T., Sano, H., 2006. Combined effect of salinomycin and feeding on whole body glucose kinetics in sheep fed a high-concentrate diet. Reprod. Nutr. Dev. 46, 503-514.

Gill, M., Siddons, R.C., Beever, D.E., 1986. Metabolism of lactic acid isomers in the rumen of silage-fed sheep. Br. J. Nutr. 55, 399-407.

Given, D.I., Rulquin, H., 2004. Utilisation by ruminants of nitrogen compounds in silage-based diets. Anim. Feed Sci. Technol. 114, 1-18.

Gleghorn, J.F., Elam, N.A., Galyean, M.L., Duff, G.C., Cole, N.A., Rivera, J.D., 2004. Effects of crude protein concentration and degradability n performance, carcass characteristics, and serum urea nitrogen concentrations in finishing beef steers. J. Anim Sci. 82, 2705-2717.

Goh, B.D., Song, Y.H., Nakanishi, Y., Manda, M., 2002. Effects of partial replacement of concentrate with soybean curd residue and Korean medical herb waste and feeding regime on nutrient digestibility and eating/resting behavior in sheep. Jpn. J. Livest. Management 37, 87-96.

Greathhead, H.M., Dawson, J.M., Schollan, N.D., Buttery, P.J., 2001. *In vivo* measurement of lipogenesis in ruminant using [1-^{14}C]acetate. Br. J. Nutr., 86, 37-44.

Hanson, R.W., Ballard, F.J., 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochem. J. 105, 529-536.

Harmon, D.L., Britton, R.A., Prior, R.L., 1983. Influence of diet on glucose turnover and rates of gluconeogenesis, oxidation, and turnover of D-(-)-lactate in the bovine. J. Nutr. 113, 1842-1850.

Harris, P.M., Skene, P.A., Buchan, V., Milne, E., Calder, A.G., Anderson, S.E., Connel, A., Lobley, G.E., 1992. Effect of food intake on hind-limb and whole-body protein metabolism in young growing steers: chronic studies based on arterio-venous techniques. Br. J. Nutr. 68, 389-407.

Hasha, G. R., 2002. Livestock Feeding and Feed Imports in the European Union: A Decade of Change. ERS No. FDS0602-01. Economic Research Service, USDA, Washington, DC. http://www.ers.usda.gov/Publications/FDS/july02/FDS0602-01/. Accessed March 12, 2012.

Herbein, J.M., Van Maaren, R.W., McGilliard, A.D., Young, J.W., 1978. Rumen propionate and blood glucose kinetics in growing cattle fed isoenergetic diets. J. Nutr. 108, 994-1001.

Hirayama, T., Hirakawa, M., Shiroma, S., 2002. Fattening performance and fatty acid composition of meat in goats fed wild grass supplemented with tofu cake. Jpn. J. Trop. Agric. 46, 183-187.

Hosoda, K., Miyaji, M., Matsuyama, H., Imai, Y., Nonaka, K., 2012. Digestibility, ruminal fermentation and methane production in Holstein steers fed diets containing soy sauce cake at 10 or 20%. Anim. Sci. J. 3, 220-226.

Huggett, A.G., Nixon, D.A., 1957. Enzymatic determination of blood glucose. J. Biochem. 66, 368-370.

Huntington, G.B., Archibeque, S.L., 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proceeding of the Am. Soc. Anim. Sci. 1-10.

Huntington, G.B., Harmon, D.L., Richards, C.J., 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84, E14-E24.

Ieki, H., Koike, M., Fujioka, K., 2010. Feed characteristics and utilization of moso-bamboo (*Phyllostachys pubescens*) pellet mixed with soybean curd residue and soy sauce cake for dairy cows. Jpn. J. Grassland Sci. 56, 34-38.

Ikeda, S., Murakami, T., 1995. Zinc chemical form in some traditional soy foods. J. Food Sci. 60, 1151-1156.

Ingle, D.L., Bauman, D.E., Garrigus, U.S., 1972. Lipogenesis in the ruminant: *in vivo* site of fatty acid synthesis in sheep. J. Nutr. 102, 617-624.

Ipharraguerre, I.R., Clark, J.H., Freeman, D.E., 2005. Varying protein and starch I the diet of dairy cows. 1. Effects on ruminal fermentation and intestinal supply of nutrients. J. Dairy Sci. 88, 2537-2555.

Judson, G.J., Anderson, E., Luick, J.R., Leng, R.A., 1968. The contribution of propionate to glucose synthesis in sheep given diets of different grain content. Br. J. Nutr. 22, 69-75.

Júnior, J.C.R., Pereira, M.N., Bôer, H., Tamingga, S., 2006. Comparison of techniques to determine the clearance of ruminal volatile fatty acids. J. Dairy Sci. 89, 3096-3106.

Kadzere, C.T., Jingura, R., 1993. Digestibility and nitrogen balance in goats given different levels of crushed whole soybeans. Small Rumin. Res. 10, 175-180.

Kajikawa, H., 1996. Utilization of by-product from food processing as livestock feed in Japan. Extensions Bulletins of Food and Fertilizer Technology Center, Taipei, pp 1-8.http://www.agnet.org/htmlarea_file/library/20110716094157/eb421.pdf. Accessed May 14, 2012.

Kakihara, A., Xu, C.C., Kudo, T., Itoh, M., Suzuki, S., Toyokawa, K., 2004. Effects of tofu cake feeding on fattening and fatty acid composition of fat tissue in lambs. Nihon Chikusan Gakkaiho 75, 17-23.

Kamata, Y., Okubo, K., Shibasaki, K., 1979. Decrease of the soybean glycinin digestibility in excess denaturation: effect of refolding. Agric. Biol. Chem. 43, 1219-1223.

Kempton, T.K., Leng, R.A., 1983. Glucose metabolism in growing lambs. Trop. Anim. Prod. 8, 244-253.

Kim, M.S., Lee, D.Y., 2010. Fermentative hydrogen production from tofu processing waste and anaerobic digester sludge using microbial consortium. Biores. Technol. 101, S48-S52.

King, K.J., Huber, J.T., Sadik, M., Bergen, W.G., Grant, A.L., King, V.L., 1990. Influence of dietary protein sources on the amino acid profiles available for digestion and metabolism in lactating cows. J. Dairy Sci. 73, 3208-3216.

Kita, K., Matsunami, S., Okumura, J., 2006. Relationship of protein synthesis to mRNA levels in the muscle of chicks under various nutritional conditions. J. Nutr. 126, 1827-1832.

Knaus, W.F., Beermann, D.H., Tedeschi, L.O., Czajkowski, M., Fox, D.G., Russell, J.B., 2002. Effects of urea, isolated soybean protein and blood meal on growing steers fed a corn-based diet. Anim. Feed Sci. Technol., 102, 3-14.

Kondo, M., Kita, K., Yokota, H., 2006. Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. Asian-Aust. J. Anim. Sci., 19, 533-540.

Köster, H.H., Cochran, R.C., Titgemeyer, E.C., Vanzant, E.S., Abdelgadir, I., St-Jean, G., 2006. Effect of increasing degradable intake protein on intake and digestion of low quality, tallgrass-prairie forage by beef cows. J. Anim. Sci. 74, 2473-2481.

Kristensen, N.B., Danfaer, A., Agergaard, N., 1998. Absorption and metabolism of short-chain fatty acids in ruminants. Arch. Anim. Nutr. 51, 165-175.

Lapierre, H., Bernier, J.F., Dubreil, P., Reynolds, C.K., Farmer, C., Ouellet, D.R., Lobley, G.E., 1999. The effect of intake on protein metabolism across the splanchnic tissues in growing beef steers. Br. J. Nutr. 81, 457-466.

Lapierre, H., Blouin, J.P., Bernier, J.F., Reynolds, C.K., Dubreuil, P., Lobley, G.E., 2002. Effect of supply of metabolizable protein on whole body and splanchnic leucine metabolism in lactating cows. J. Dairy Sci., 85, 2631-2641.

Lee, S.D., Williams, W.F., 1962. Acetate turnover rate in the bovine. J. Dairy Sci. 45, 893-896.

Lindgreen, S.E., Axelsson, L.T., McFeeters., 1990. Anaerobic $_{L}$-lactate degradation by *Lactobacillus plantarum*. FEMS Microbiol. Lett. 66, 209-214.

Linington, M.J., Meyer, J.H.F., van der Walt, J.G., 1998. Rumen VFA production rates, whole body metabolite kinetics and blood hormone concentrations in sheep fed high- and low-fibre diets. S. Afr. J. Anim. Sci. 28, 82-98.

Liu, S.M., Lobley, G.E., MacLeod, N.A., Kyle, D.J., Chen, X.B., Ørskov, E.R., 1995. Effects of long-term protein excess or deficiency on whole-body protein turnover in sheep nourished by intragastric infusion of nutrients. Br. J. Nutr. 73, 829-839.

Lobley, G.E., 1992. Control of the metabolic fate of amino acids in ruminants: a review. J. Anim. Sci. 70, 3264-3275.

Lobley, G.E., 2003. Protein turnover: what does it mean for animal production?. Can. J. Anim. Sci. 83, 327-340.

Lobley, G.E., Connel, A., Buchan, V., 1987. Effect of food intake on protein and energy metabolism in finishing beef steers. Br. J. Nutr. 57, 457-465.

Ma, C.Y., Liu, W.S., Kwok, K.C., Kwok, F., 1996. Isolation and characterization of protein from soymilk residue (okara). Food Res. Int. 29, 799-805.

MAFF., 2009. Ministry of Agriculture, Forestry and Fisheries Research Council. Animal feed situation in Japan. MAFF, Tokyo, 22 pp. www.maff.go.jp/j/chikusan/kikaku/lin/l_siryo/pdf/meguru_siryou.pdf. Accessed May 14, 2012.

Martin, C., Kristensen, N.B., Huhtanen, P., 2001. Comparison of non-tracer and tracer methods for determination of volatile fatty acid production rate in the rumen of sheep fed two levels of intake. Br. J. Nutr. 86, 331-340.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal Nutrition. 7th Ed. Pearson, UK.

Mlay, P.S., Pereka, A.E., Weisbjerg, M.R., Hvelplund, T., Madsen, J., 2003. Digestion and passage kinetics of fibre in mature dairy heifers maintained on poor quality hay as affected by the source and level of nitrogen supplementation. Anim. Feed Sci. Technol. 109, 19-33.

Moreau, N.M., Goupry, S.M., Antignac, J.P., Monteau, F.J., Le Bizec, B.J., Champ, M.M., Martin, L.J., Dumon, H.J., 2003. Simultaneous measurement of plasma concentrations and ^{13}C-enrichment of short-chain fatty acids, lactic acid and ketone bodies by gas chromatography coupled to mass spectrometry. J. Chromatogr. B. 784, 395-403.

NARO., 2009. Standard tables of feed composition in Japan. National Agriculture and Food Research Organization (NARO). Japan Livestock Industry Association.

Nielsen, K., Kondrup, J., Elsner P., Juul, A., Jensen, E.S., 1994. Casein and soybean protein have different effects on whole body protein turnover at the same nitrogen balance. Br. J. Nutr. 72, 69-81.

Niwa, Y., Nakanishi, G., 1995. Research on the utilization of food by-product to growing and finishing pigs: The effects of tofu cake silage feeding on growth and body fat. Jpn. J. Swine Sci. 32, 1-7.

NRC, 1985. Nutrient Requirements of Sheep, 6th revised edition. National Academy Press, Washington, DC.

Ohno, A., Ano, T., Shoda, M., 1996. Use of soybean curd residue, okara, for the solid-satte substrate in the production of a lipopeptide antibiotic iturin A, by *Bacillus subtilis* NB22. Process. Biochem. 31, 801-806.

Ohshima, M., McDonald, P., 1978. A review of the changes in nitrogenous compounds of herbage during ensilage. J. Sci. Food Agric. 29, 497-505.

Oltjen, R.R., Putnam, P.A., 1966. Plasma amino acid and nitrogen retention by steers fed purified diets containing urea or isolated soy protein. J. Nutr. 89, 385-390.

Ørskov, E.R., Grubb, D.A., Wenham, G., Corrigall, W., 1979. The sustenance of growing and fattening ruminants by intragastric infusion of volatile fatty acid and protein. Br. J. Nutr. 41, 553-558.

Ortigues-Marty, I., Vernet, J., Majdoub, L., 2003. Whole body glucose turnover in growing and non-productive adults ruminants: meta-analysis and review. Reprod. Nutr. Dev. 43, 371-383.

O'Toole, D., 1999. Characteristics and use of okara, the soybean residue from soymilk production- A Review. J. Agric. Food Chem. 47, 363-371.

Peripolli, V., Prates, E.R., Barcellos, J.O.J., Neto, J.B., 2011. Fecal nitrogen to estimate intake and digestibility in grazing ruminants. Anim. Feed Sci. Technol. 163, 170-176.

Ponnampalam, E.N., Egan, A.R., Sinclair, A.J., Leury, B.J., 2005. Feed intake, growth, plasma glucose and urea nitrogen concentration, and carcass traits of lambs fed isoenergetic amounts of canola meal, soybean meal, and fish meal with forage based diet. Small Rumin. Res. 58, 245-252.

Prior, R.L., 1976. Effects of dietary soy or urea nitrogen and feeding frequency on nitrogen metabolism, glucose metabolism and urinary metabolite excretion in sheep. J. Anim. Sci. 42, 160-167.

Rashad, M.M., Mahmoud, A.E., Abdou, H.M., Nooman, M.U., 2011. Improvement of nutritional quality and antioxidant activities of yeast fermented soybean curd residue. Afr. J. Biotech. 10, 5504-5513.

Robinson, P.H., Tamminga, S., Van Vuuren, A.M., 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livest. Prod. Sci. 15, 173-189.

Rocchiccioli F., Leroux J.P., Cartier, P., 1981. Quantitation of 2-ketoacids in biological fluids by gas chromatography chemical ionization mass spectrometry of *o*-trimethylsilyl-quinoxalinol derivatives. Biomed. Mass Spectrom. 8, 160-164.

Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J., Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70, 3551-3561.

Sabine, J.R., Johnson, B.C., 1964. Acetate metabolism in the ruminant. J. Biol. Chem. 239, 89-93.

Sano, H., Kajita, M., Fujita, T., 2004. Effect of dietary protein intake on plasma Leucine flux, protein synthesis, and degradation in sheep. Comp. Biochem. Physiol. 139B, 163-164.

Sano, H., Konno, S., Shiga, A., 2000. Chromium supplementation does not influence glucose metabolism or insulin action in response to cold exposure in mature sheep. J. Anim. Sci. 78, 2950-2956.

Sano, H., Shibasaki, S., 2011. Effects of urea and soybean meal supplementation on rates of plasma leucine turnover and protein synthesis in sheep fed concentrate-based diets. J. Appl. Anim. Res. 39, 239-244.

Sano, H., Shibasaki, S., Sawada H., 2009. The effect of the source of nitrogen supplementation on nitrogen balance, rates of plasma leucine turnover, protein synthesis and degradation in sheep. Arch. Anim. Nutr. 63, 401-412.

Sarwar, M., Firkins, J.K., Eastridge, M.L., 1992. Effects of varying forage and concentrate carbohydrates on nutrient digestibilities and milk production by dairy cows. J. Dairy Sci. 75, 1533-1542.

SAS., 1996. SAS/STAT® software: changes and enhancement through Release 6.11. SAS Institute Inc. Cary. NC, USA.

Savary, I.C., Hoskin, S.O., Dennison, N., Lobley, G.E., 2001. Lysine metabolism across the hindquarters of sheep; effect of intake on transfers from plasma and red blood cells. Br. J. Nutr. 85, 565-573.

Savary-Auzeloux, I., Hoskin, S.O., Lobley, G.E., 2003. Effect of intake on whole body plasma amino acid kinetics in sheep. Reprod. Nutr. Dev. 43, 117-129.

Schelling, G.T., Hinds, F.C., Hatfield, E.E., 1967. Effect of dietary protein levels, amino acid supplementation and nitrogen source upon the plasma free amino acid concentrations in growing lambs. J. Nutr. 92, 339-347.

Schmidt, S.P., Keith, R.K., 1983. Effects of diet and energy intake on kinetics of glucose metabolism in steers. J. Nutr. 113, 2155-2163.

Schroeder, G. F., Titgemeyer, E. C., Awawdeh, M. S., Smith, J. S., Gnad, D. P., 2006. Effect of energy level on methionine utilization by growing steers. J. Anim. Sci. 84, 1497-1504.

Seal, C.J., Parker, D.S., Avery, P.J., 1992. The effect of forage and forage-concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric- and portal-drained viscera in growing steers. Br. J. Nutr. 67, 355-370.

Song, M.K., Kennelly, J.J., 1989. In situ degradation of feed ingredients, fermentation pattern and microbial populations as influenced by ruminal ammonia concentration. Can. J. Anim. Sci. 69, 999-1006.

Sugiura, K., Yamatani, S., Watahara, M., Onodera, T., 2009. Ecoofed, animal feed produced from recycle food waste. Vet. Italiana 45, 397-404.

Sultan, J.I., Loerch, S.C., 1992. Effects of protein and energy supplementation of wheat straw-based diets on site of nutrient digestion and nutrient metabolism of lambs. J. Anim. Sci. 70, 2228-2234.

Sutton, J.D., 1989. Altering milk composition by feeding. J. Dairy Sci. 72, 2801-2814.

Swanson, K.C., Caton, J.S., Redmer, D.A., Burke, V.I., Reynolds, L.P., 2000. Influence of undegraded intake protein on intake, digestion, serum hormones and metabolites, and nitrogen balance in sheep. Small Rumin. Res. 35, 225-233.

Taillandier, D., Guezennec, C.Y., Patureau-Mirand, P., Bigard, X., Arnal, M., Attaix, D., 1996. A high protein diet does not improve protein synthesis in the nonweight-bearing rat tibialis anterior muscle. J. Nutr. 126, 266-272.

Takahashi, T., Horiguchi, K., Goto, M., 2005. Effect of crushing unhulled rice and the addition of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of whole crop rice silage, and its digestibility and rumen fermentation status in sheep. Anim. Sci. J. 76, 353-358.

Taylor, K.A.C.C., 1996. A simple colorimetric assay for muramic acid and lactic. Appl. Biochem. Biotech. 56, 49-58.

Trenkle, A., Kuhlemeier, K.V., 1966. Relationship of rumen volatile acids, blood glucose, and plasma nonesterified fatty acids in sheep. J. Anim. Sci. 25, 111-1115.

Tserng, K.Y., Kalhan, S.C., 1983. Estimation of glucose carbon recycling and glucose turnover with [U-^{13}C]glucose. Am. J. Physiol. 245, E476-E482.

Ulyatt, M.J., Whitelaw, F.G., Watson, F.G., 1970. The effect of diet on glucose entry rates in sheep. J. Agric. Sci. 75, 565-570.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Veenhuizen, J.J., Russel, R.W., Young, J.W., 1988. Kinetics of metabolism of glucose, propionate and CO_2 in steers as affected by injecting phlorizin and feeding propionate. J. Nutr. 118, 1366-1375.

Wang, F., Nishino, N., 2008. Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. J. Dairy Sci. 91, 2380-2387.

Waterlow, J.C. 2006. Protein turnover. CAB International, UK.

Weatherburn, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39, 971–974.

Wohlt, J.E., Sniffen, C.J., Hoover, W.H., Johnson, L.L., Walker, C.K., 1976. Nitrogen metabolism in wethers as affected by dietary protein solubility and amno acid profile. J. Anim. Sci. 42, 1280-1289.

Wolfe, R.R., Chinkes, D.L., 2005. Isotope Tracers in Metabolic Research. Principles and Practice of Kinetic Analysis. 2nd ed. Wiley, US.

Xu, C.C., Suzuki, H., Toyokawa, K., 2001. Characteristics of ruminal fermentation of wethers fed tofu cake silage with ethanol. Anim. Sci. J. 72, 299-305.

Yang, C.M.J., 2005. Soybean milk residue ensiled with peanut hulls: fermentation acids, cell wall composition, and silage utilization by mixed rumen microorganisms. Biosource Tech. 96, 1419-1424.

Yilmaz, Z., Atinok, S., Kocabas, Z., 2009. An investigation on quality parameters of the silages made by corn and soybean grown in different seeding rates. J. Anim. Vet. Adv. 8, 1856-1859.

Yokota, T., Ohami, H., Ohishi, H., Hattori, T., Watanabe, K., 1996. Repression of acute gastric mucosal lesions by antioxidant-containing fraction from fermented products of okara (bean-curd residue). J. Nutr. Sci. Vitaminol. 42, 167-172.

Yost, W.M., Young, J.W., Schmidt, S.P., McGilliard, A.D., 1977. Gluconeogenesis in Ruminants: Propionic acid production from a high-grain diet fed to cattle. J. Nutr. 107, 2036-2043.