Chapter-3

Effects of feeding garlic stem and leaf on turnover rates of plasma acetate and glucose in sheep

Introduction:

In previous studies, feeding effects of garlic stem and leaf have been investigated on N metabolism kinetics in sheep (Chapter -1 and Chapter -2). It seemed that garlic stem and leaf might have stimulating effects on N metabolism in sheep. The ruminant animals have evolved a specially adapted digestive system and most of the dietary carbohydrate is fermented to VFA and therefore, glucose required for the body must be supplied from gluconeogenesis. Therefore, acetate and glucose are both important blood metabolites influenced by nutritional status in animal body. In ruminants, acetate is the main VFA varying from approximately 75 to 40 % of total molar proportions (Bergman, 1990) and acetate alone can fulfill about 50% of daily energy requirements for sheep (Skutches et al., 1979). It has been reported that garlic components affect ruminal fermentation in cattle (Cardozo et al., 2005; Busquet et al., 2006). Glucose metabolism in the liver was also influenced by garlic components (Chang and Johnson, 1980). Thus, it was hypothesized that garlic stem and leaf could enhance acetate and glucose metabolism in ruminants. Therefore, the current study was conducted to access the feeding effects of garlic stem and leaf on plasma acetate and glucose metabolism in sheep using isotope dilution methods of [1-^{13}C]Na acetate and [U-^{13}C]glucose .

Materials and methods:

The experiment was conducted from July to August, 2011. Before starting,

experimental procedures and protocol were approved according to the guidelines of the Animal Care Committee of Iwate University.

Animals, diets and experimental protocol

Four wethers (Corriedale x Suffolk crossbred, 41.5±1.6 kg of initial BW, 1 to 1.5 years of age) were used. The experiment was performed using a crossover design with two 21 days periods, each consisting of 14 days of adaptation and 7 days of sampling. The sheep were penned in individual stalls during the adaptation period and then moved to environmental controlled house at an air temperature of 23℃, 70% relative humidity and with lighting from 08.00 to 22.00 h and maintained in individual metabolic crates. Throughout the experiment, wethers were fed either mixed hay (Hay-diet, CP 9.9%, NDF 68.6%; on air DM basis) of orchardgrass (*Dactylis glomerata*) and reed canarygrass (*Phalaris arundinacea*) or hay plus garlic stem and leaf at a ratio of 9:1 (GL-diet, CP 9.8%, NDF 65.8%; on air DM basis). The amount of hay was calculated according to Agricultural and Food Research Council (AFRC, 1993) at maintenance ME level. The animals were received 7.4 g CP and 120 kcal ME/kg $BW^{0.75}$/d for the Hay-diet. Feed were offered in two meals daily at 08:30 and 18:30 h and had free access to water. The garlic stem and leaf were collected from Aomori Prefecture in Japan and stored in room freezer at -30℃. Before feeding, frozen samples were brought to room temperature, cut into small pieces and offered to animals. The sheep were weighed on the day of starting experiment and every 7 days intervals.

Collection of rumen fluid

On the 20th day of each sampling period, to characterize the ruminal fermentation patterns, ruminal fluid was collected with a stomach tube and a vacuum pump at 0 (immediately before feeding), 2 and 4 h after feeding. The pH value of the rumen fluid

was measured immediately with a pH meter (F-51, Horiba Electronics Ltd., Japan). From the total sample, a portion was centrifuged at 8000 rpm for 10 min at 2℃ (RS-18IV, Tomy, Japan) and the supernatant was acidified with 0.1 N HCl for determining NH_3 and urea concentrations and remaining was used for rumen VFA analysis. Finally, both the samples were stored at -30℃ for later analysis.

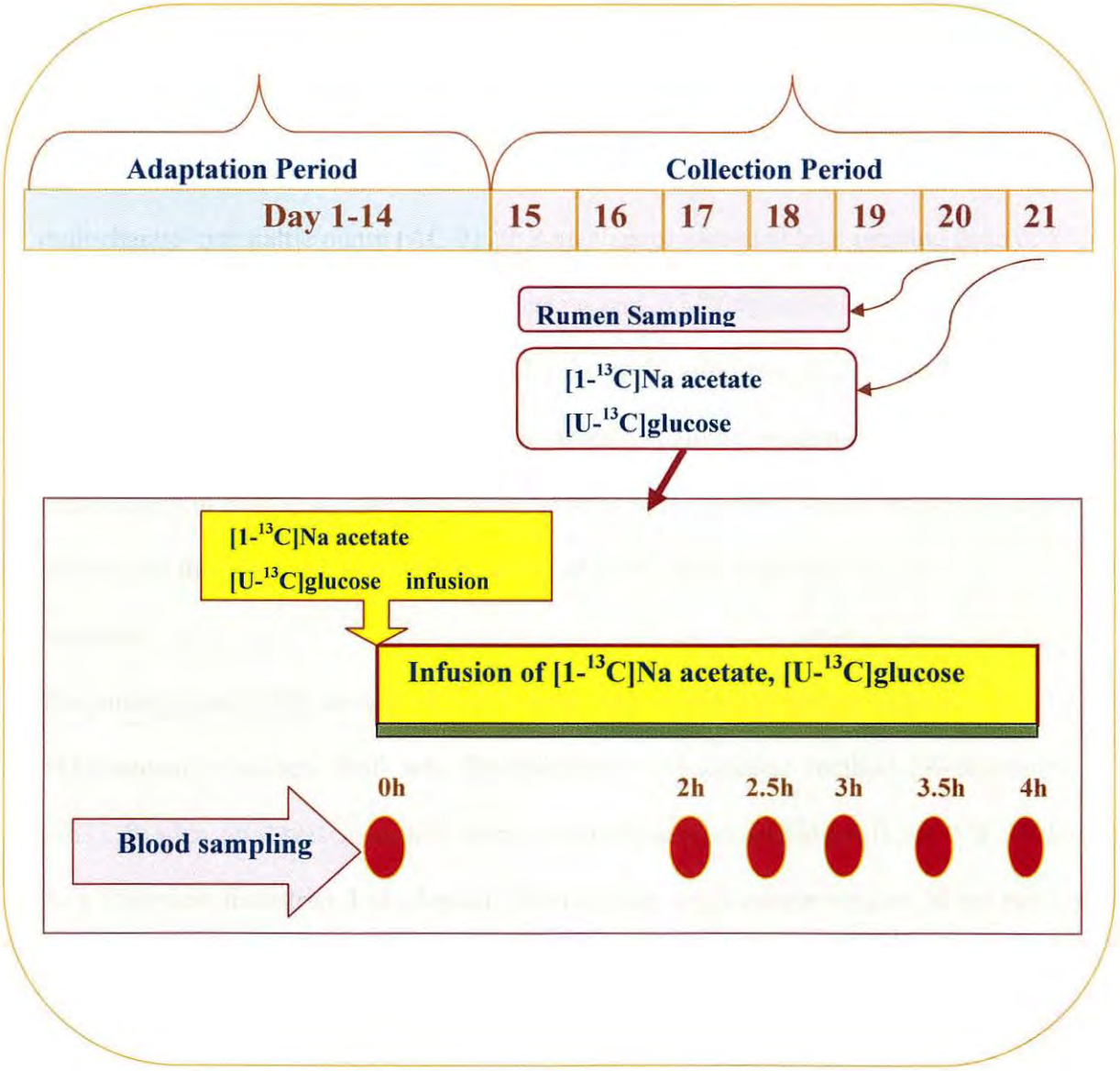

Figure 3.1. Experimental layout and isotope infusion protocol.

Isotope dilution method

An isotope dilution method using [1-^{13}C]Na acetate and [U-^{13}C]glucose was accomplished on day 21th of each dietary treatment to determine turnover rates of plasma acetate and glucose in sheep. Isotope dilution protocol is shown in Figure 3.1. On the morning of isotope dilution method, wethers were fitted with temporary right and left jugular vein catheters, one for isotope infusion and another for blood sampling. Both the catheters were filled with 3.8 % trisodium citrate solution. At 12.00 h, on the respective day, [1-^{13}C]Na acetate and [U-^{13}C]glucose were infused, via infusion catheter, at the rates of 87 and 2.9 μmol/kg $BW^{0.75}$/h respectively for 4 h (12.00 to 16.00 h) by a multichannel peristaltic pump (AC-2120, Atto, Japan) preceded by priming dose of 87 and 2.9 μmol/kg $BW^{0.75}$ of [1-^{13}C]Na acetate and [U-^{13}C]glucose respectively. Blood samples were collected just before (10 mL) and at 30 min intervals (5 mL) over the last 2 h of isotope infusion. Blood collected in tubes containing sodium heparin was placed immediately in chilled ice and then centrifuged at 8000 rpm for 10 min at 2℃ to harvest plasma. All the harvested plasma was stored at -30℃ until analyzed.

Analyses

The nitrogen and NDF content of feeds were determined accordingly (Chapter-1). The NH_3 content of rumen fluid was determined by colorimetric method (Weatherburn, 1967). Rumen urea was measured using a commercial available kit (Urea NB, Wako Pure Chemical Industries Ltd., Japan). Volatile fatty acids concentrations in the rumen fluid were determined through gas chromatography (HP-5890, Hewlett Packard, USA) as described previously in Chapter-1. Plasma free amino acids, NH_3 and urea concentrations were determined after deproteinization with SSA by automated amino acid analyzer (JLC-500/V, JEOL, Japan) described briefly in Chapter- 1. Concentrations

of plasma NEFA were enzymatically determined using a diagnostic kit (NEFA C, Wako Pure Chemicals Industries Ltd., Japan).

Plasma [1-^{13}C]acetate enrichments and concentrations of plasma acetate and lactate were determined by the procedure of Moreau et al. (2003) using the gas chromatography mass spectrometry with selection ion monitoring. Briefly, 1 mL blood plasma was mixed with 4% SSA and 100 µL of solution containing two internal standards of 2-ethylbutyric acid (0.5 mmol/L) and 4-methylvaleric acid (0.5 mmol/L) for the purpose of deproteinization and kept into refrigerator for 30 min. After centrifugation (0℃, 12000 rpm, 10 min) the resulted supernatant was transferred into screw capped glass tube and acidified with 25 µL of 37% HCl. After acidification, 2 mL diethyl ether was added into the vials and shacked vigorously and centrifuged (1000 rpm, 3 min). Then the upper organic layer was transferred into new glass tubes and again ethyl extraction was performed on the aqueous layer. This double extraction procedure was performed for three times with same amount of diethyl ether. After complete recovery of the organic layer, 0.1 mL of the extract was mixed with 20 µL MTBSTFA and kept at room temperature for 1h. Finally, enrichments were measured using gas chromatography mass spectrometry. The ions monitored for the molecules are: m/z 117 and 118.

Plasma glucose enrichment was determined using gas chromatography mass spectrometry (QP-2010, Shimadzu, Japan) with selection ion monitoring according to the procedures of Tserng and Kalhan, (1983). In brief, 1 mL blood plasma was added with 4 % SSA and kept into refrigerator for 30 min. After centrifugation (0℃, 12000 rpm, 10 min) twice, the supernatant was passed through both cation exchange resin (Dowex 50 X8 (H^+ form, 200-400 mesh, 0.5 mL) and anion exchange resin (Dowex 1

X8 (CH_3COO^- form, 200-400 mesh, 1 mL) and washed twice with 0.5 mL distilled water. Then 0.1 mL of supernatant transferred into glass screw capped tube and placed in dessication until drying. After drying, 0.5 mL pyridine was added with it, heated at 90℃ for 30 min, and again added with 0.5 mL of acetic anhydride. Again, it was heated at 90℃ for 1h, kept into chilled ice for 30 min, mixed with 2 mL water and 1 mL chloroform, shacked vigorously for 1 min and removed upper layer. Thereafter, it was mixed with 2 mL water, shacked vigorously for 1 min and upper part removed and centrifuged (0℃, 3000 rpm, 5 min) again and removed upper part. Finally, lower organic portion was transferred into small glass tube, dried at 30℃ by N_2 gas and kept into dessicator. After drying, mixed with 100 μL chloroform and taken into chromatographic vials for analysis of glucose enrichment. The ions determined are: m/z 314 and m/z 319. Concentration of plasma glucose was determined by the method of Huggett and Nixon (1957).

Calculations

Plasma acetate and glucose turnover rates were calculated as follows (Tserng and Kalhan, 1983) :

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

where, I is the infusion rate of [1-^{13}C]Na acetate and [U-^{13}C]glucose and E is the plasma isotopic enrichments of [1-^{13}C]acetate and [U-^{13}C]glucose at steady state respectively.

Statistical analysis

All data were analysed by ANOVA using the MIXED procedure of SAS (1996) for a crossover design. Animal was designed as the random effect and diet and period as the fixed effect for all analyses. The Tukey adjustment was used for the time course of changes. Results were considered significant at the $P < 0.05$ level, and a tendency was

defined as $0.05 \leqq P < 0.10$.

Results:

Time course of changes in rumen pH and principal VFA concentrations are shown in Figure 3.2. Average ruminal fermentation patterns are presented in Table 3.1. Rumen fluid pH was almost same between treatments. Rumen NH_3 and urea concentrations also did not differ between the diets. Rumen total VFA concentration as well as concentrations of acetate, isobutyrate, butyrate and isovaterate tended to be higher ($P < 0.10$) in GL-diet than Hay-diet. In addition, concentrations of propionate and valerate were significantly higher ($P = 0.03$ and $P = 0.04$ respectively) and acetate to propionate ratio was significantly lower ($P = 0.03$) for GL-diet than Hay-diet.

Among plasma free amino acids determined at the pre-infusion period of the isotope dilution method, concentration of plasma glutamic acid was significantly lower ($P = 0.04$) and concentration of plasma tryptophan was significantly higher ($P = 0.02$) in GL-diet than Hay-diet (Table 3.2). Concentrations of plasma NEFA and lactate did not differ between the diets.

Time course of changes in concentrations of plasma acetate and enrichments of plasma [1-^{13}C]acetate are given in Figure 3.3. Time course of changes in concentrations of plasma glucose and enrichments of plasma [U-^{13}C]glucose are also given in Figure 3.4. Plasma acetate turnover rate tended to be higher ($P = 0.06$) in GL-diet than Hay-diet (Table 3.3). Plasma acetate concentration did not differ between the diets. Concentrations and turnover rates of plasma glucose did not differ between the diets.

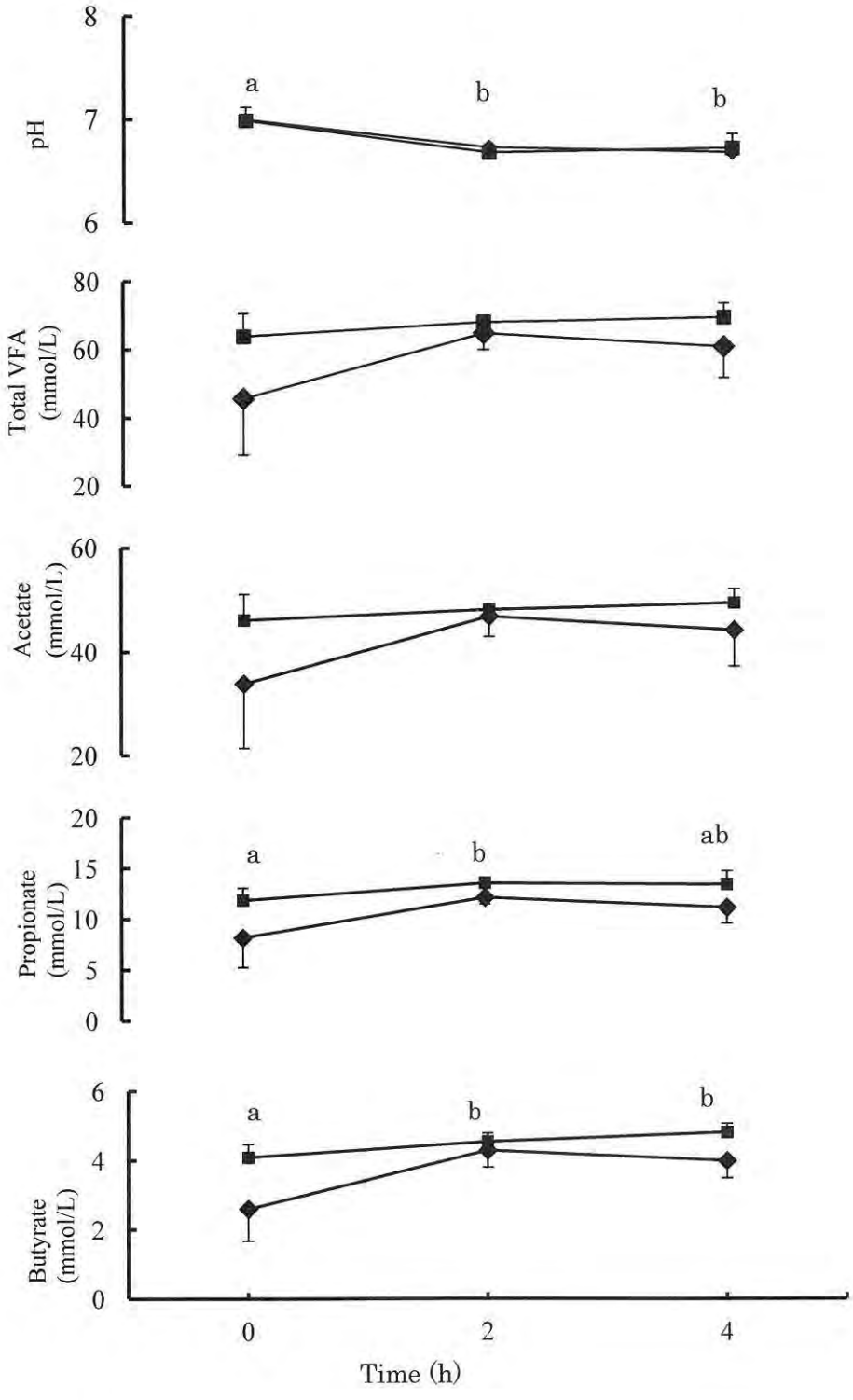

Figure 3.2. Time course of changes in rumen pH and VFA concentrations in sheep (Hay-diet, —◆— and GL-diet, —■—). a, b- differ significantly ($P < 0.05$).

Discussion:

The rumen pH value was not altered by offering garlic stem and leaf in GL-diet. In our present study, ruminal fermentation patterns in GL-diet suggested that garlic stem and leaf might have stimulating effects on ruminal VFA concentrations in sheep. There is substantial evidence that herbal plant extracts may affect microbial fermentation (Busquet et al., 2005a, 2005b, 2006; Cardozo et al., 2005) in the rumen in cattle. Cardozo et al. (2005) found that garlic extract (3 and 30 mg/L of culture fluid) significantly increased total VFA concentration to an *in vitro* culture system.

Table 3.1 Effects of feeding garlic stem and leaf on ruminal fermentation patterns in sheep[a].

	Treatment[b]			
Items	Hay-diet	GL-diet	SEM[c]	*P*-value
pH	6.82	6.79	0.04	0.38
NH_3 (mmol/L)	5.0	5.4	0.5	0.54
Urea (mmol/L)	3.9	4.2	0.3	0.44
Total VFA (mmol/L)	57.2	67.1	2.6	0.07
Individual VFA (mmol/L)				
Acetate	41.6	47.9	1.8	0.08
Propionate	10.5	12.9	0.6	0.03
Isobutyrate	0.5	0.6	0.03	0.08
Butyrate	3.6	4.5	0.2	0.07
Isovalerate	0.6	0.8	0.05	0.07
Valerate	0.3	0.4	0.02	0.04
Acetate: propionate	4.0	3.7	0.08	0.03

[a] Values represent means of four sheep.

[b] Hay-diet, mixed hay of orchardgrass and reed canarygrass hay; GL-diet, hay plus garlic stem and leaf (at a ratio of 9:1).

[c] SEM, standard error of the mean.

Table 3.2 Effects of feeding garlic stem and leaf on plasma free amino acids, NH_3, urea, glucose and NEFA concentrations at the pre-infusion period in sheep[a].

Items	Treatment[b]		SEM[c]	*P*-value
	Hay-diet	GL-diet		
Essential amino acids (μmol/L)				
Threonine	219	241	19	0.56
Valine	322	335	9	0.55
Methionine	25	27	2	0.36
Isoleucine	113	121	4	0.50
Leucine	168	173	5	0.68
Phenylalanine	72	87	4	0.11
Histidine	59	55	2	0.23
Lysine	147	167	13	0.44
Non essential amino acids (μmol/L)				
Serine	136	170	18	0.35
Asparagine	103	116	12	0.57
Glutamic acid	100	93	5	0.04
Glutamine	291	287	16	0.88
Glycine	524	586	31	0.97
Alanine	246	235	13	0.16
Tyrosine	88	92	5	0.59
Tryptophan	49	69	4	0.02
Arginine	187	223	10	0.14
Proline	124	130	7	0.76
Ammonia (μmol/L)	176	180	2	0.30
Urea (mmol/L)	6.64	6.71	0.08	0.62
Glucose (mmol/L)	3.42	3.60	0.09	0.41
NEFA (μEq/L)	190	210	10	0.31
Lactate (μmol/L)	236	377	15	0.25

[a] Values represent means of four sheep.

[b] Hay-diet, mixed hay of orchardgrass and reed canarygrass hay; GL-diet, hay plus garlic stem and leaf (at a ratio of 9:1).

[c] SEM, standard error of the mean.

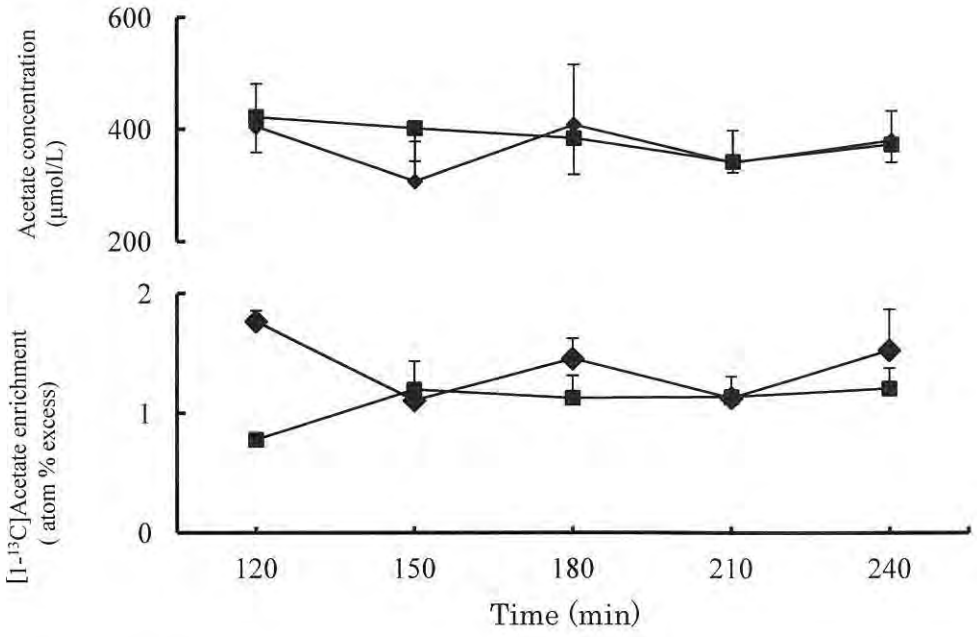

Figure 3.3.Time course of changes in concentrations and enrichments of plasma [1-^{13}C]acetate in sheep (Hay-diet, ◆ and GL-diet, ■).

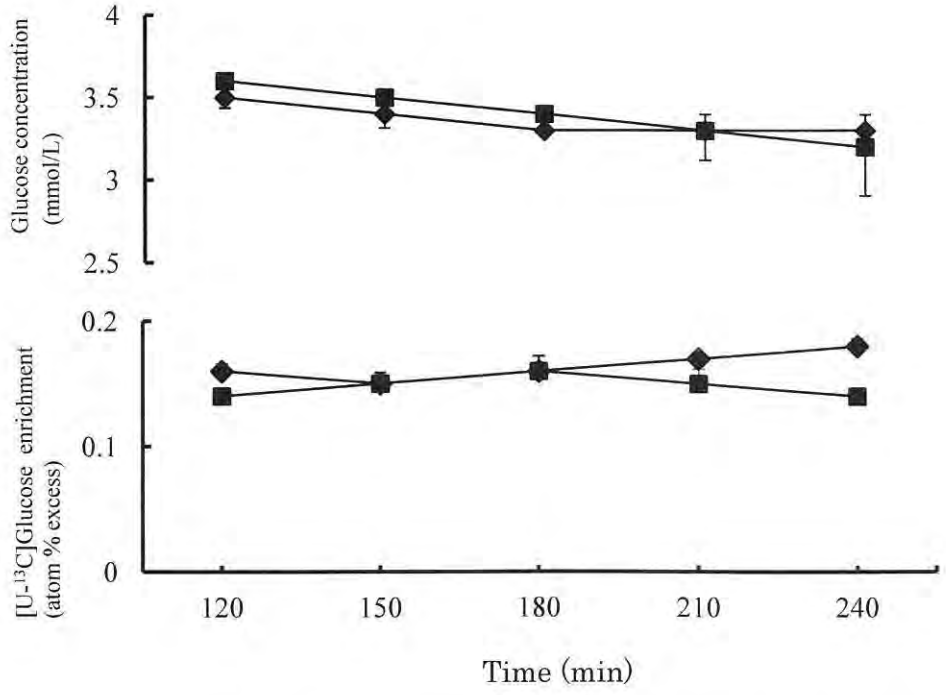

Figure. 3.4. Time course of changes in concentrations and enrichments of plasma [U-^{13}C]glucose in sheep (Hay-diet, ◆ and GL-diet, ■).

Table 3.3 Feeding garlic stem and leaf on turnover rates (GluTR, AceTR) and concentrations of plasma acetate and glucose in sheep[a].

Items	Treatment[b]		SEM[c]	*P*-value
	Hay-diet	GL-diet		
AceTR (mmol/kg $BW^{0.75}$/h)	5.15	6.49	0.65	0.06
Acetate (μmol/L)	367	385	30	0.14
GluTR (mmol/kg $BW^{0.75}$/h)	1.39	1.57	0.05	0.13
Glucose (mmol/L)	3.42	3.41	0.01	0.85

[a] Values represent means of four sheep.

[b] Hay-diet, mixed hay of orchardgrass and reed canarygrass hay; GL-diet, hay plus garlic stem and leaf (at a ratio of 9:1).

[c] SEM, standard error of the mean.

Busquet et al. (2005a, 2005b) also studied garlic oil and four of its compounds on ruminal microbial fermentation using an *in vitro* batch culture system and observed that garlic components resulted in lower acetate and higher propionate and butyrate proportions than control. Garlic stem and leaf supplementation resulted in a reduction of acetate to propionate ratio which was consistent with previous studies (Busquet et al., 2005a, 2005b; Cardozo et al., 2005). The decreased in the acetate: propionate observed in GL-diet is consistent with the fermentation profile commonly found in methane inhibitors (Busquet et al., 2005b; Cardozo et al., 2005). Garlic bioactive components present in garlic stem and leaf might stimulate microbial activity and microbial proliferation in rumen and resulted in higher VFA concentrations in sheep fed with GL-diet than Hay-diet. In my previous findings (Chapter-1) garlic stem and leaf silage did not affect total rumen VFA concentration as well as concentrations of individual

VFA. But in this study, fresh garlic stem and leaf resulted higher total as well as individual VFA concentrations in sheep. The inconsistencies among the studies might be due to garlic components varying in bioactive components as because fresh garlic stem and leaf might retain higher amounts of bioactive components.

Until now, to our knowledge, responses of garlic constituents on plasma acetate and glucose metabolism are not well investigated either in human or in animal studies. Al-Mamun (2008), examined the effects of plantain herb on plasma acetate metabolism in sheep using [1-^{13}C]Na acetate infusion method in sheep and found numerically higher value of plasma AceTR in plantain herb group than the control group. In another study, feeding effects of Hop (*Humulus lupulus*) on acetate metabolism in sheep was investigated and it was found that hop residues did not affect plasma AceTR in sheep (Al-Mamun et al., 2009). Although both diets were around isonitrogenous and isoenergetic, the tendency of increase in plasma AceTR might be due to higher availability of acetate, happening from increased acetate production in the rumen and effect of plenty of bioactive components in GL-diet. Al-Mamun et al. (2007) determined the effects of plantain herb using [6, 6-^{2}H]glucose isotope dilution method and did not find significant effects of plantain herb on plasma glucose metabolism in sheep. Al-Mamun (2008) also determined the effect of plantain herb using [U-^{13}C]glucose isotope dilution method to know the responses of plasma glucose metabolism to exogenous insulin infusion in sheep and plantain herb did not affect glucose turnover rate in sheep. Nevertheless, it was also reported that glucose metabolism in the liver and insulin secretion was significantly increased by garlic components (Chang and Johnson, 1980).

Chapter-4

Summary and conclusions:

Antibiotic growth promoters have been practiced in animal industry since it's discovery in the 1940s. But in recent years, the use of feed additives in animal feeds is facing reduced social acceptance due to the increasing public concerns about the bad residual effects of antibiotics on human health. The European Union (EU) and other developed countries have taken widespread steps to ban antibiotics as feed additives. In particular, the use of antibiotics as growth promoters has been banned in the EU since January 2006 (OJEU, 2003). Consequently, it becomes the challenge for animal scientists to explore alternatives of antibiotics growth promoters for animal industry. For this reason, attention has recently shifted to various herbs and aromatic plants. Garlic (*Allium sativum*) is one of the medicinal plants which have been paid a lot of interests throughout human history as a medicinal panacea in almost every known culture due to it's antiviral, antifungal, antiparasitic, antioxidant, anticancer, immune enhancing and liver functioning activities. It has a complex mixture of many secondary plant products including alliin, allicin, diallyl sulfide and diallyl disulfide. During harvesting, only garlic bulb is used for human consumption in various purposes and remaining residues consisted of stem, leaf and flowers have no commercial use and are simply thrown or disposed. The aim of the present study was to know the feeding effects of garlic stem and leaf on ruminal fermentation characteristics, nitrogen (N) balance, microbial N supply (MNS) and the intermediate nutrients metabolism in sheep which ultimately introduce it as a potential ruminant feeds towards coming centuries.

Initially, an experiment was conducted to assess the feeding effects of garlic stem and leaf silage on N balance, ruminal fermentation patterns, rates of plasma leucine

turnover (LeuTR), whole body protein synthesis (WBPS) and degradation (WBPD) in sheep using an isotope dilution method of [1-^{13}C]Leu (Experiment 1). The experimental diets fed in this trial consisted of mixed hay (Hay-diet, as control) and hay plus garlic stem and leaf silage (GS-diet, at ratio of 9:1) in a crossover design for each 21 day period. Diets were formulated at maintenance ME level and were around isoenergetic and isonitrogenous. The isotope dilution method using [1-^{13}C]Leu was performed on the 21st day of each dietary treatment for measuring plasma LeuTR. Nitrogen digestibility, N absorption and N retention did not differ between the diets. Ruminal total volatile fatty acids (VFA) concentration as well as concentrations of principal VFA did not differ between the diets. Plasma non-esterified fatty acids (NEFA) concentration was significantly lower ($P < 0.01$) and glucose concentration was greater ($P = 0.03$) for GS-diet compared with Hay-diet. Feeding garlic silage did not influence plasma LeuTR, WBPS and WBPD in sheep.

It was assumed that during ensiling process some nutrients and bioactive components of garlic stem and leaf might be lost. Furthermore, for better understanding about the feeding effects of garlic stem and leaf on N metabolism in sheep, 2nd experiment was conducted to assess the feeding effects of fresh garlic stem and leaf on turnover rates of plasma phenylalanine (PheTR), tyrosine (TyrTR) and WBPS in sheep using an isotope dilution method of [$^{2}H_5$]Phe and [$^{2}H_2$]Tyr (Experiment 2). Effects of feeding garlic stem and leaf on MNS also studied. The sheep were fed either mixed hay (Hay-diet, as control) or hay plus garlic stem and leaf diet (GL-diet) at ratio of 9:1. The experiment was performed using a crossover design with two 21 day periods. The isotope dilution method using [$^{2}H_5$]Phe and [$^{2}H_2$]Tyr was performed on the 21st day of each dietary treatment to measure the turnover rates of Phe and Tyr in sheep. Nitrogen

intake did not differ between the diets and N absorption and N digestibility were higher ($P < 0.05$) in GL-diet than Hay-diet. Plasma PheTR tended to be higher ($P = 0.06$) during GL feeding and TyrTR did not differ between the diets. Further, WBPS tended to be greater ($P = 0.05$) for the GL-diet compared with the Hay-diet. Total purine derivatives excretion and MNS were higher ($P < 0.05$) in GL-diet than Hay-diet. A positive relationship ($R^2 = 0.58$, $P < 0.05$) was found between MNS and WBPS in the study.

In the succeeding study, isotope dilution methods using [1-^{13}C]Na acetate and [U-^{13}C]glucose was simultaneously performed to know the feeding effects of garlic stem and leaf on plasma acetate and glucose metabolism in sheep (Experiment 3). The sheep were offered either mixed hay (Hay-diet) or hay plus garlic stem and leaf (GL-diet, in a 9:1 ratio) in a crossover design for 21 day period. The isotope dilution method using [1-^{13}C]Na acetate and [U-^{13}C]glucose was performed on the 21st day of each period to measure turnover rates of plasma acetate and glucose in sheep. Concentration of ruminal total VFA as well as concentrations of acetate and butyrate tended to be higher ($P < 0.10$) in GL-diet than Hay-diet. Further, propionate concentration was significantly higher ($P = 0.03$) for GL-diet. Turnover rate of plasma acetate tended to be higher ($P = 0.06$) for GL-diet than Hay-diet and that of glucose did not differ between the diets.

To the best of my knowledge, it was the first work using garlic stem and leaf as a ruminant feed. The present study showed some noble findings using garlic stem and leaf as ruminant feed. Ruminal fermentation characteristics, N balance, MNS and WBPS were influenced by garlic stem and leaf feeding. Further, garlic stem and leaf might have stimulating effects on plasma acetate metabolism in sheep.

This investigation therefore suggested that garlic stem and leaf can be used as a potential alternative feed source for ruminants and this supplementation will facilitate the proper use of the residue as well as reduce feed scarcity and minimizing environmental pollution. In addition, further investigations can be performed using garlic stem and leaf in ruminants.

Summary in Japanese:

畜産業では1940年代から抗生物質が実用化されてきた。しかしながら、最近、人の健康に対する抗生物質残留の懸念から、飼料添加物の使用は社会的許容を失いつつある。ヨーロッパ連合(ＥＵ)や他の先進国では飼料添加物としての抗生物質の使用が制限されており、特に、ＥＵでは2006年１月から全面的に使用が禁止された。その結果、畜産学に携わる研究者たちは抗生物質に替わる新たな成長促進剤の開発に着手した。このような背景から種々のハーブやアロマ植物が注目されている。ニンニク(*Allium sativum*)は、alliin、allicin、diallyl sulfide、diallyl disulfideなどの二次代謝産物を豊富に含み、抗ウィルス作用、抗菌作用、抗酸化作用、抗癌作用、免疫賦活作用、肝機能強化作用など、様々な機能を有するため、古くから知られている薬草の一つである。ニンニク球根は種々の目的に利用されるが、花、茎、葉部には市場価値がなく、廃棄されている。本研究の目的は、ニンニク茎葉部の有効利用のため、ヒツジにおいてルーメン発酵、窒素(Ｎ)出納、微生物態Ｎ供給量(MNS)および体内の栄養素代謝に及ぼすニンニク茎葉給与の影響を明らかにし、反芻家畜の飼料として確立することである。

はじめに、ヒツジのＮ出納、ルーメン発酵、血漿ロイシン代謝回転速度(LeuTR)、全身のタンパク質合成速度(WBPS)および分解速度(WBPD)に及ぼすニンニク茎葉サイレージ給与の影響を測定した（実験１）。実験飼料は混播牧草区(Hay区)および牧草＋ニンニク茎葉サイレージ区(9:1、ＧＳ区)とし、１期21日のクロスオーバー法にしたがって実施した。給与量は両飼料区とも維持量の代謝エネルギー(ME)とし、ほぼ同等のME、粗タンパク質(CP)を給与した。血漿LeuTRを測定するため、それぞれの飼料区21日目に[1-^{13}C]Leuの同位元素希釈法を実施した。Ｎ消化率、Ｎ吸収およびＮ蓄積は飼料間に差がなかった。ルーメン内総揮発性脂肪酸（VFA）および主要VFA濃度は飼料間に差がなかった。血漿遊離脂肪酸（NEFA）濃度はHay区と比較してＧＳ区が低く($P < 0.01$)、血漿グルコース濃度は高かった($P = 0.03$)。ニンニク茎葉給与は血漿LeuTR、WBPSおよびWBPDに影響を与えなかった。

サイレージ調製期間中にニンニク茎葉の栄養素および生理活性成分が失われると推測される。そこで、ヒツジに生のニンニク茎葉を添加した飼料を給与し、[$^{2}H_5$]Pheおよび[$^{2}H_2$]Tyrの同位元素希釈法を用いて血漿フェニルアラニンおよびチロシン代謝回転速度(PheTR、TyrTR)およびWBPSを測定した（実験２）。MNSに及ぼすニンニク茎葉給与の影響も測定した。混播牧草(Hay区)あるいは牧草＋生のニンニク茎葉(9:1、ＧＬ区)を給与した。実験は１期21日間のクロスオーバー法にて実施した。血漿PheTR、TyrTRを測定するため、それぞれの飼料区21日目に[$^{2}H_5$]Pheおよび[$^{2}H_2$]Tyrの同位元素希釈法を実施した。Ｎ摂取量は飼料間に差がなく、Ｎ吸収量およびＮ消化率はHay区よりＧＬ区が高かった($P < 0.05$)。総プリン誘導体排泄量およびMNSはHay区よりＧＬ区が高かった($P < 0.05$)。血漿PheTRはＧＬ区が高い傾向を示し($P = 0.06$)、TyrTRは飼料間で差がなかった。さらに、WBPSはHay区と比較してＧＬ区が高い傾向を示した($P = 0.05$)。

実験３では、ヒツジの血漿酢酸およびグルコース代謝に及ぼすニンニク茎葉給与の影響を明らかにするために、[1-^{13}C]酢酸 Na および[U-^{13}C]グルコースの同位元素希釈法を実施した。混播牧草(Hay 区)あるいは牧草＋ニンニク茎葉(9:1、ＧＬ区)を給与し、実験は１期 21 日のクロスオーバー法にしたがって実施した。血漿酢酸およびグルコース代謝回転速度を測定するため、それぞれの飼料区 21 日目に[1-^{13}C]酢酸 Na および[U-^{13}C]グルコースの同位元素希釈法を実施した。ルーメン総ＶＦＡ、酢酸および酪酸濃度は Hay 区よりＧＬ区が高い傾向を示し（$P < 0.10$）、プロピオン酸濃度は有意に高かった（$P = 0.03$）。血漿酢酸代謝回転速度は Hay 区よりもＧＬ区が高い傾向を示し（$P = 0.06$）、血漿グルコース代謝回転速度は飼料間に差がなかった。

反芻家畜の飼料としてニンニク茎葉を使用した研究はこれが最初であり、いくつかの貴重な知見を得た。ルーメン発酵特性、Ｎ出納、MNS および WBPS はニンニク茎葉給与によって影響された。さらに、ニンニク茎葉はヒツジの血漿酢酸代謝を刺激する効果を有するかもしれない。

したがって、本研究の結果は、ニンニク茎葉が反芻家畜における有望な代替飼料となり得ること、ニンニク茎葉添加は飼料不足の軽減、環境汚染の最小限化と同時に農産廃棄物の適切使用を促進することを示した。さらに、反芻家畜においてニンニク茎葉を用いたさらなる研究を実施すべきであろう。

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"The educated differs from the uneducated as much as the living differs from the dead."

Aristotle

The End……