Effect of grinding process of steam-rolled barley grain on plasma glucose and leucine kinetics and protein synthesis in sheep

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ABSTRACT
An isotope dilution method of [U-13C]glucose and [l-13C]leucine was simultaneously conducted to determine the effect of grinding process of steam-rolled barley grain on rates of plasma glucose and leucine turnover and whole-body protein synthesis (WBPS) in sheep. Six adult sheep were assigned to two dietary treatments and the experiment utilized a crossover design for two 21-day periods. The experimental diet was consisted of mixed hay and steam-rolled barley grain (1:1 ratio). Barley grain was treated either with or without grinding (GR diet and SR diet, respectively). Sheep were fed isoenergetically and isonitrogenously in both the treatments. The primed-continuous infusion method of [U-13C]glucose and [l-13C]leucine was simultaneously performed on day 21. The pH and ammonia concentrations in rumen fluid decreased (P < 0.05) and concentrations of total VFA, acetate, propionate and n-butyrate increased (P < 0.05) after feeding. Rates of plasma glucose and leucine turnover and WBPS did not differ between the dietary treatments. It is likely that grinding process of steam-rolled barley grain did not influence plasma glucose and leucine kinetics and WBPS in sheep fed the diets of mixed hay and barley grain at 1:1 ratio.

1. Introduction
In ruminants, microorganisms in the rumen produce volatile fatty acids (VFA) from carbohydrates such as cellulose and starch. Ruminant animals, the host animals, absorb VFA mainly from the rumen wall and utilize VFA as the major energy source. Therefore, quantity and quality of diets and production rate or molar ratio of VFA in the rumen are closely related to intermediary metabolism in ruminants (Sutton et al. 2003). To clarify the relationship, researches were conducted by changing sources of diets, roughage: concentrate ratio, and by providing VFA salt supplementation to diets and VFA infusions into the rumen (Harmon et al. 1983; Gross et al. 1990; Fujita et al. 2006; Sano et al. 2008). However, these methodologies involve in factors of different dietary sources, supplements or infusates. Grinding process of grain feeds was reported to influence performance, nutrient digestibility and rumen characteristics in sheep (Ørskov et al. 1974; Abdul-Razzaq et al. 1988; Theurer et al. 1999). Abdul-Razzaq et al. (1988) reported that in growing lambs the grinding process of whole loose barley produced an acetic acid type of fermentation. Therefore, it was hypothesized that grinding process of grain feeds influenced intermediary metabolism through digestive and rumen fermentable functions in sheep. However, relatively few information about grinding process of grain feeds on intermediary metabolism has been available in sheep (Abdul-Razzaq and Bickerstaffe 1989; Landau et al. 1992; Kiran and Mutsvangwa 2007). The objectives of this experiment were to determine the effect of grinding of steam-rolled barley grain on concentrations of plasma metabolites and rates of plasma glucose and leucine turnover in addition to N balance and rumen fermentable functions in sheep fed the diets of mixed hay and barley grain at 1:1 ratio.

2. Materials and methods
2.1. Animals and diets
All experimental procedures were reviewed and approved by the Animal Care Committee of Iwate University. The experiment was carried out without any noticeable stress to the animals. Six crossbred (Corriedale × Suffolk) shorn sheep (4 ewes and 2 wethers), aged 1–2 years and weighing 35 ± 1 kg of BW were used. The experiment utilized a crossover design with two 21-day periods. Animals were housed in individual pens in an animal room during the preliminary period and the first 2 weeks of the experiment. Then, the sheep were moved to metabolic cages in a controlled environment chamber at an air temperature of 23 ± 1°C, with 70% RH and lighting present from 0800 to 2200. The experimental diet consisted of mixed hay of orchardgrass and reed canary grass (ME 1.79 kcal/g, 9.6% CP as fed basis) and steam-rolled barley grain (ME 2.74 kcal/g, 10.0% CP as fed basis) at 1:1 ratio based on a fed basis, as shown in Table 1 (NRC 1985). The barley grain was treated either with or without grinding (GR and SR diets, respectively). The dietary treatments were designed to be same ME intake and CP intake. Three sheep were fed the GR diet during the first period, and then fed the SR diet during...
the second period. The other three sheep were subjected to these dietary treatments in reverse order. The animals were fed once daily at 1400, and commonly consumed their ration within 1 h. Drinking water was available ad libitum. The sheep were weighed on the start of the experiment, on day-15 and after each dietary treatment.

### 2.2. Nitrogen balance test and rumen fluid collection

Nitrogen (N) balance was determined over the successive 5 days (day 16 to day 20) in each dietary treatment. Faeces were collected for each 24 h, dried in a forced air oven (60°C, 48 h), and weighed after placing at room temperatures for 5 days. An aliquot was ground (Cyclotec 1093 Sample Mill, Foss, Sweden) and stored at room temperatures, until further analysis. Urine was collected for each 24 h in a bottle containing 100 ml of 6 N H2SO4 and total volume was recorded. Aliquots of urine samples were stored at −30°C pending analysis. On day 20 of each dietary treatment, rumen fluid (30 ml) was collected through a stomach tube at 0 (immediately before feeding), 1, 2, 3, 4, 5 and 6 h after feeding. Rumen fluid was centrifuged at 1000×g for 10 min at 4°C (RS-18IV, Tomy, Japan) and an aliquot supernatant was mixed with 0.1 mol/l HCl and residual supernatant were stored at −30°C until ammonia and VFA determinations, respectively.

### 2.3. Isotope dilution methods

On day 21 of each dietary treatment, the isotope dilution method of [U-13C] glucose and [1-13C] leucine was simultaneously conducted to determine turnover rates of plasma glucose and leucine, respectively. Catheters for infusion and blood sampling were inserted into both jugular veins on the morning of each determination of the isotope dilution method. The catheters were filled with a sterile solution of 0.13 mol/l trisodium citrate. At 1200 on the day of the isotope dilution method, 2.6 µmol kg BW−0.75 of [U-13C]glucose (δ-Glucose, 13C6, 99%, Cambridge Isotope Laboratories, Inc., USA) and 6 µmol kg BW−0.75 of [L-13C]leucine (L-Leucine, 1-13C, 99%; Cambridge Isotope Laboratories, Inc.) dissolved in 10 ml of saline solution (0.15 mol/l sodium chloride) were injected into the jugular catheter as the priming dose. Then, [U-13C]glucose and [L-13C]leucine dissolved in saline solution were continuously infused at rates of 2.6 and 6 µmol kg BW−0.75 h−1, respectively, by a multichannel peristaltic pump (AC-2120, Atto Co. Ltd., Japan) through the same catheter for 4 h. Blood samples (5 ml) were taken immediately before and at 30-min intervals during the last 90 min of isotope infusion. Samples were transferred into centrifuge tubes containing heparin sodium, stored temporally in ice and were centrifuged at 8000×g for 10 min at 4°C. The plasma was separated and stored at −30°C until further analyses.

### 2.4. Analyses

Enrichments of plasma [U-13C]glucose were determined with gas chromatography mass spectrometry (GC/MS, OP-2010, Shimadzu, Japan) by the procedures of Tsemg and Kalhan (1983) with slight modification as described previously (Sano et al. 1996). Concentrations of plasma glucose were enzymatically determined by the method of Huggett and Nixon (1957). Concentrations of plasma free amino acids and α-ketoisocapric acid (α-KIC) and enrichments of plasma α-[L-13C]KIC were determined with the GC/MS by the procedures of Moreau et al. (2003). Concentrations of plasma NEFA were enzymatically determined using the diagnostic kit (NEFA C test, Wako, Japan). Concentrations of VFA in the rumen fluid were determined with gas chromatography (HP-5890, Hewlett Packard, USA) after steam distillation, as described previously (Sano et al. 2009). Ammonia concentrations in rumen fluid were determined by a colorimetric method (Weatherburn 1967). Nitrogen contents in diets, faeces and urine were also determined by the colorimetric method after Kjeldahl digestion.

### 2.5. Calculations

Mean values with SEM are given. Turnover rates of plasma glucose (GTR) and leucine (LeuTR) were calculated using the equation by Wolfe (1984).

\[
TR = I \times \left(\frac{1}{E} - 1\right),
\]

where \(I\) is the infusion rate of each stable isotope and \(E\) is the corresponding plasma isotope enrichment during the steady states. Plasma α-KIC enrichments were used for calculation of LeuTR as described previously (Sano et al. 2009). Whole-body protein synthesis (WBPS) was calculated from the equation as described by Schroeder et al. (2006).

\[ WBPS = \frac{\text{LeuTR}}{\text{(Leucine concentration in carcass protein)}} - \text{(urinary N excretion)}} \times 6.25. \]

Leucine concentrations in carcass protein were estimated to be 6.6% (Harris et al. 1992).

### 2.6. Statistics

Data were analysed with the MIXED procedure of the SAS (1996). The crossover design was used to test for effects of period, diet and the interaction. The random effect was sheep. The significant period and interaction effect were not detected in the parameters determined except for the period effect in some plasma amino acid concentrations. Therefore, only diet effect was considered. Repeated statement was used for the time course of the parameters and the difference

| Treatmenta | GR diet | SR diet |
|------------|--------|--------|
| Mixed hay (g kg−0.75 d−1) | 27.9 | 27.9 |
| Barley (g kg−0.75 d−1) | 27.9 | 27.9 |
| MEb (kcal kg−0.75 d−1) | 126 | 126 |
| CP (g kg−0.75 d−1) | 5.4 | 5.4 |

Note: Values presented on a fed basis.

aGR diet = Mixed hay plus ground steam-rolled barley grain; SR diet = Mixed hay plus steam-rolled barley grain.

bEstimated from the AFRC (1993).
in the least square means with the Tukey’s adjustment was used, if the time effect was significant. Results were considered significant at the \( P < 0.05 \) level. The tendency was defined as \( 0.05 \leq P < 0.10 \).

3. Results

Nitrogen intake, N excretion into urine and N retention remained unchanged between diets (Table 2). Nitrogen digestibility was slightly lower for the GR diet compared with the SR diet due to the trend of greater faecal N excretion \( (P = 0.18) \), but the difference was not significant \( (P = 0.17) \). The pH of rumen fluid decreased \( (P < 0.05) \) after feeding and reached the minimal values at 5 h after feeding (6.0 and 6.1 for the GR and SR diets, respectively; Figure 1). Concentrations of total VFA, acetate, propionate and n-butyrate in rumen fluid increased \( (P < 0.05) \) after feeding. Ammonia concentrations in rumen fluid decreased \( (P < 0.05) \) gradually after feeding. No time-relative trends of plasma glucose and \( \alpha \)-KIC concentrations, and plasma \([U-13C]glucose\) and \( \alpha-[1-13C]KIC \) enrichments were detected \( (P > 0.05) \) during the latter periods of the isotope dilution method (Figure 2). Plasma glucose concentrations and GTR did not differ \( (P = 0.82 \) and \( P = 0.32, \) respectively) between the diets (Table 3). Plasma \( \alpha \)-KIC concentrations, plasma LeuTR and WBPS were similar \( (P = 0.99, \) \( P = 1.00 \) and \( P = 0.96, \) respectively) between the GR and SR diets. Concentrations of plasma lactate and NEFA at the pre-infusion period did not differ \( (P = 0.60 \) and \( P = 0.17, \) respectively) between the dietary treatments (Table 4). Plasma leucine and proline concentrations at the pre-infusion period were higher \( (P = 0.04 \) and \( P = 0.02, \) respectively) and plasma valine and glycine concentrations tended to be higher \( (P = 0.05 \) and \( P = 0.06, \) respectively) for the GR diet compared with the SR diet. Concentrations of other plasma amino acids did not differ between the diets.

4. Discussion

4.1. Digestive functions

Processing of barley grain influenced digestive functions in lactating dairy cows and growing lambs (Yang et al. 2000; Kiran and Mutsvangwa 2007; Gozho et al. 2008). Slightly lower N digestibility for the GR diet compared with the SR diet observed in the present experiment was in agreement with the results by Kiran and Mutsvangwa (2007), who reported that N digestibility was lower in growing lambs fed pelleted barley compared with those fed dry-rolled barley. Yang et al. (2000) reported that in lactating dairy cows fed barley grain-based diets, both total and post-ruminal digestibilities of starch and CP were inversely related to processing index due to increased particle outflow rate from the rumen, although barley grain processing did not influence ruminal digestibility of organic matter. Gozho et al. (2008) reported that in lactating dairy cows, even though methods of barley grain processing altered urea N entry into the gastrointestinal tract, utilization of recycled urea N for microbial protein was unaffected. Unchanged N digestibility and N retention in both diets would be responsible to feed these diets isoenergetically. Nitrogen retention would be more related to ME intake compared with N intake, as reported by Fujita et al. (2006).

4.2. Rumen fermentable functions

Yang et al. (2000) reported that in lactating cows, barley grain processing influenced pH, acetate, propionate and acetate: propionate ratio in rumen fluid. Although grain processing had

### Table 2. Effect of grinding process of barley grain on nitrogen (N) balance and digestibility in sheep.

| Treatments | GR diet | SR diet | SEM  | \( P \)-value |
|------------|---------|---------|------|---------------|
| No. of sheep | 6       | 6       |      |               |
| N intake (g kg\(^{-0.75}\) d\(^{-1}\)) | 0.870 | 0.870 | 0.0003 | 0.54 |
| N in faeces (g kg\(^{-0.75}\) d\(^{-1}\)) | 0.302 | 0.276 | 0.012 | 0.18 |
| N in urine (g kg\(^{-0.75}\) d\(^{-1}\)) | 0.356 | 0.348 | 0.022 | 0.81 |
| N retention (g kg\(^{-0.75}\) d\(^{-1}\)) | 0.212 | 0.246 | 0.024 | 0.31 |
| N digestibility (%) | 65.3 | 68.3 | 1.4 | 0.17 |

\( ^{a} \text{GR diet = Mixed hay plus ground steam-rolled barley grain; SR diet = Mixed hay plus steam-rolled barley grain.} \)
less impact on starch digestion by sheep than cattle (Theurer 1986), processing of barley, corn, oats and wheat influenced rumen pH, total VFA, acetate and propionate concentrations, especially acetate: propionate molar ratio was lower for ground pelleted diets compared with whole loose diets in lambs (Ørskov et al. 1974). Ørskov and Fraser (1975) also reported that in sheep rumen pH and acid detergent fibre digestibility of roughage reduced greater for pelleted barley-based diet compared with whole barley-based diet. Therefore, it is probable that processing of grain diets regulated rumen characteristics; even same source and amount of diets are used. Abdul-Razzaq et al. (1988) reported that in growing sheep fed the diet contained 85% barley grain, grinding process of barley grain yielded acetate-type fermentation in the rumen, whereas feeding whole loose barley produced a propionate type of rumen fermentation. Propionate-type fermentation increased plasma insulin and glucose concentrations, reduced plasma branched-amino acid concentration, and increased fat and protein deposition. Moreover, Bengochea et al. (2005) studied ruminal fermentation characteristics in steers fed the diet contained 40% barley grain and found that grinding process did not effect on total VFA concentration and individual VFA molar ratio, whereas ruminal pH responded quadratically. Gozho et al. (2008) reported that in lactating dairy cows fed 30% barley grain diet, the total VFA, acetate and butyrate concentrations in the rumen were lower for pelleted barley compared with dry-rolled barley, whereas propionate and valerate concentrations were higher. In the present experiment, similar trends were observed although the differences were not significant. Therefore, the effect of barley grain processing on rumen fermentable functions may be influenced by the barley grain content of the diet.

### 4.3. Kinetics of plasma glucose and leucine and plasma metabolite concentrations

In the present experiment observed normal concentrations of plasma glucose, lactate, NEFA and amino acids were observed to be the normal range. Landau et al. (1992) reported that in ewes grinding or extruding, processing of corn grain decreased plasma glucose entry rate. In the present experiment, plasma GTR remained unchanged with grinding process of barley grain. It is well-known that physiological and nutritional conditions influence plasma glucose metabolism. Therefore, unchanged plasma glucose turnover rate may be due to the appropriate intake of the present experiment. The numerical data in the present result were comparable with the data obtained in sheep fed alfalfa hay cube and commercial concentrate (Sano and Fujita 1988) reported that in growing sheep fed the diet contained 85% barley grain, grinding process of barley grain yielded acetate-type fermentation in the rumen, whereas feeding whole loose barley produced a propionate type of rumen fermentation. Propionate-type fermentation increased plasma insulin and glucose concentrations, reduced plasma branched-amino acid concentration, and increased fat and protein deposition. Moreover, Bengochea et al. (2005) studied ruminal fermentation characteristics in steers fed the diet contained 40% barley grain and found that grinding process did not effect on total VFA concentration and individual VFA molar ratio, whereas ruminal pH responded quadratically. Gozho et al. (2008) reported that in lactating dairy cows fed 30% barley grain diet, the total VFA, acetate and butyrate concentrations in the rumen were lower for pelleted barley compared with dry-rolled barley, whereas propionate and valerate concentrations were higher. In the present experiment, similar trends were observed although the differences were not significant. Therefore, the effect of barley grain processing on rumen fermentable functions may be influenced by the barley grain content of the diet.

| Treatments | GR diet | SR diet | SEM | P-value |
|------------|---------|---------|-----|---------|
| No. of sheep | 6 | 6 | | |
| Glucose | 4.0 | 4.0 | 0.2 | 0.82 |
| Turnover rate | 2.5 | 2.3 | 0.1 | 0.32 |
| Leucine | 8.2 | 8.3 | 1.2 | 0.99 |
| Turnover rate | 0.40 | 0.40 | 0.04 | 1.00 |
| WBPS (g kg⁻¹ | 0.69 | 0.70 | 0.07 | 0.96 |

GR diet = Mixed hay plus ground steam-rolled barley grain; SR diet = Mixed hay plus steam-rolled barley grain.

| Treatments | GR diet | SR diet | SEM | P-value |
|------------|---------|---------|-----|---------|
| No. of sheep | 6 | 6 | | |
| Lactate | 0.50 | 0.48 | 0.02 | 0.60 |
| NEFA | 0.10 | 0.16 | 0.04 | 0.17 |
| Histidine | 40 | 29 | 14 | 0.51 |
| Isoleucine | 73 | 72 | 5 | 0.87 |
| Leucine | 106 | 92 | 10 | 0.04 |
| Lysine | 55 | 50 | 13 | 0.67 |
| Methionine | 10 | 10 | 2 | 0.93 |
| Phenylalanine | 36 | 34 | 3 | 0.37 |
| Threonine | 247 | 214 | 96 | 0.77 |
| Valine | 221 | 192 | 18 | 0.05 |
| Alanine | 189 | 174 | 12 | 0.17 |
| Aspartic acid | 1.2 | 1.4 | 0.6 | 0.79 |
| Glutamic acid | 18 | 20 | 9 | 0.85 |
| Glycine | 664 | 578 | 57 | 0.06 |
| Proline | 137 | 116 | 16 | 0.02 |
| Serine | 117 | 115 | 42 | 0.97 |
| Tyrosine | 35 | 40 | 16 | 0.78 |
| Tryptophan | 14 | 14 | 2 | 0.72 |

GR diet = Mixed hay plus ground steam-rolled barley grain; SR diet = Mixed hay plus steam-rolled barley grain.

Data at the pre-infusion period only.

Figure 2. Time course changes of plasma glucose and α-KIC concentrations, and the enrichments of [U-13C]glucose and α-[1-13C]KIC during the last 1.5 h of isotope dilution techniques.
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Hay and barley grain at 1:1 ratio. Glucose and leucine metabolism in sheep fed the diet of mixed process of steam-rolled barley grain did not influence plasma involved in the inconsistent results. In conclusion, grinding by different barley grain processing influenced little on WBPS but tyrosine oxidation was higher for acetic acid type of fermentation compared with propionic acid type of fermentation in growing lambs (Abdul-Razzaq and Bickerstaffe 1989). The unchanged WBPS with grinding process agreed with the data obtained in the present experiment. Lobley et al. (1996) reported that whole-body Leu flux was unaltered either by diet type or ammonia salt infusion. They also found that the Leu move-ments to WBPS, calculated based on enrichments of either Leu or 4-methyl-2-oxopentanoate (MOP), were also uninfluenced by treatments. Whole-body Leu oxidation was significantly lower on mixture of grass and barley pellets compared with grass pellets although fractional oxidations of the MOP-based flux were similar across treatments. Plasma amino acid concentrations were presented as determined by the GC/MS and a total of 16 amino acids were detected. Most of them were comparable with the values determined by the automated amino acid analyzer. In the present experiment, grinding process of barley grain did not influence intermediary metabolism of plasma glucose and leucine and WBPS in sheep. This may be related that the low level of steamed-rolled barley to the experimental diets (50% of diets) did not affect rumen fermentation and N digestibil-ity markedly. In this regard, Abdul-Razzaq and Bickerstaffe (1989) reported that in growing lambs pelleted ground barley (85% of diets) produced an propionic acid type of fermentation. The acetate concentration in the rumen fluid had the similar trend, but propionate concentration was similar in both diets. The dietary component and the amount of barley used may be involved in the inconsistent results. In conclusion, grinding process of steam-rolled barley grain did not influence plasma glucose and leucine metabolism in sheep fed the diet of mixed hay and barley grain at 1:1 ratio.

Disclosure statement

No potential conflict of interest was reported by the authors.

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