

Metabolism and glucose kinetics in sheep fed plantain and orchard grass and exposed to cold

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Abstract To investigate the characteristics of plantain (PL), a forage herb, as a feed for ruminants, chemical components, energy digestibility, rumen constituents, concentrations of blood metabolites (glucose, nonesterified fatty acids, lactate, acetate, and propionate), and insulin were measured in four sheep fed PL and orchard grass (OR), a forage. Net blood glucose turnover rate in response to cold exposure (0–4°C on Day 5) was also determined using an isotope dilution method of [U-¹³C]glucose. Dry matter intake was numerically lower for the PL diet than for the OR diet (942 versus 1154 g day⁻¹), and was lower ($P = 0.03$) during cold exposure than in the thermoneutral environment (1009 versus 1087 g day⁻¹). Energy digestibility was similar between the PL and OR diets. No significant differences in blood metabolite and insulin concentrations in plasma were detected between the PL and OR diets. Net blood glucose turnover rate was also similar between the diets. The rate increased ($P = 0.002$) during cold exposure, with no significant diet and environment interaction. The forage herb PL seems to be comparable to OR as regards digestive and metabolic characteristics and blood glucose metabolism of sheep.

Keywords herb; cold exposure; glucose metabolism; stable isotope; sheep

INTRODUCTION

The narrow leaf plantain (PL), *Plantago lanceolata* L., is a perennial herb and is established as a diet for ruminants. Plantain is known to contain a range of chemical compounds with anti-bacterial, anti-inflammatory, and anti-tumour properties. Therefore, it is expected that the forage herb PL may moderate a variety of stresses in ruminants and may influence performance. Cold exposure is one of the stresses which reduce the productivity of ruminants through modified digestive, metabolic, and endocrine functions (Kennedy et al. 1976; Weekes et al. 1983; Young et al. 1989). However, information is not available relating to digestive and metabolic responses and intermediary metabolism in ruminants fed PL and exposed to a cold environment. The objective of this experiment was to investigate the digestive and metabolic characteristics of PL as a ruminant diet by comparison with another forage, orchard grass (OR, *Dactylis glomerata* L.). Moreover, blood glucose turnover rate was measured using an isotope dilution procedure with [U-¹³C]glucose infusion in both thermoneutral and cold environments.

MATERIALS AND METHODS

Animals and diets

Four crossbred (Corriedale × Suffolk) shorn sheep (two ewes and two rams) aged 2–4 years and weighing 46 ± 4 kg were used. The sheep were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery. Animals were housed in individual metabolic cages in a controlled environment chamber at an air temperature of 20°C. The dietary treatment consisted of PL or OR. The experiment used a cross-over design with two 3-week periods. Sheep were assigned to the two groups according to sex, age, and body weight. Two

sheep were fed the PL diet for 3 weeks during the first period, and then they were fed the OR diet for 3 weeks during the second period. The other two sheep were fed the diets in the reverse order. Animals were given either diet, which was freshly harvested in the morning, once daily at 1200 h. Plantain was harvested at the bolting stage and OR at the heading stage. Water was available *ad libitum*. The sheep were weighed once a week throughout the preliminary and experimental periods and on the day before the initiation of cold exposure. A catheter for infusion was inserted into a jugular vein on the day before the blood sampling for daily profiles in the thermoneutral environment, and was maintained until the end of the determination of blood glucose kinetics in a cold environment. A catheter for blood sampling was inserted into the skin loop of the carotid artery on the morning of each determination of blood glucose metabolism. Catheters were filled with 38 g litre⁻¹ of trisodium citrate sterile solution. After determinations in the thermoneutral environment were completed, sheep were shorn closely again and then exposed to a cold environment (0°C from 1000 to 2200 h, 4°C from 2200 to 1000 h) for 5 days. Determinations of the blood samples and blood glucose kinetics were conducted as in the thermoneutral environment. The surgery, management, and blood sampling were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Experimental procedures

The total faecal collection was carried out over 4 successive days (Days 10–13) in the thermoneutral environment for determination of energy digestibility. Blood (10 ml) was withdrawn from the jugular catheter at 0900 h for 4 days in the thermoneutral (Days 13–16) and cold environments (Days 18–21) respectively, for determinations of blood metabolite and hormone concentrations. Prefeeding rumen fluid samples were also collected by stomach tube immediately after blood sampling on Days 14 and 15.

The isotope dilution procedure for determination of net blood glucose turnover rate was carried out on Day 16 of the thermoneutral environment and on Day 21 which corresponded to the fifth day of cold exposure. At 1100 h, 10 mg of [U-¹³C]glucose (D-glucose-¹³C, 99 atom % excess ¹³C; Isotec Inc., A Matheson, USA Co., United States) dissolved in 10 ml of saline solution (9 g litre⁻¹ of sodium chloride solution) was injected into the jugular

catheter as a priming dose. [U-¹³C]glucose (300 mg litre⁻¹ in 9 g litre⁻¹ of sodium chloride solution) was then continuously infused by a multichannel peristaltic pump (AC-2120, Atto Co. Ltd., Japan) at a rate of 8 µg kg^{-0.75} of body weight per min through the same catheter for 5 h. Blood samples (6 ml) were taken from the carotid artery catheter immediately before and 1, 2, 3, 3.5, 4, 4.5, and 5 h after the initiation of [U-¹³C]glucose infusion. Sheep were fed 1 h after the initiation of the infusion, therefore the last 2-h period of the isotope dilution method corresponded to 3–4 h after feeding. Blood samples were transferred into centrifuge tubes containing sodium heparin and were chilled until centrifugation.

Chemical analyses

Energy in diets and faeces was determined by an automatic bomb calorimeter (CA-4, Shimadzu, Japan). Chemical compounds of diets were analysed by the AOAC (1990) method. Potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) contents in the diets were determined by an atomic absorption spectrometer (Z-8100, Hitachi Ltd., Japan). Blood samples for daily profiles and the isotope dilution procedure were centrifuged at 8000 g for 10 min at 4°C (RS-18IV, Tomy, Japan), and the plasma was stored at -25°C until further analyses. Concentrations of glucose in plasma were determined with an automated glucose analyser (GLU-1, DKK-TOA Co., Japan). Derivatisation of plasma glucose before determination of isotopic abundance was performed by the procedure of Tserng & Kalhan (1983) with slight modifications as described previously (Sano et al. 1996). The isotopic abundance of the glucose derivative was determined with a gas chromatography-combustion-isotope ratio mass spectrometry (DELTA^{plus}, ThermoQuest, Germany). Plasma insulin concentrations were measured with a RIA kit (IRI "Eiken", Eiken Chemical Co. Ltd., Japan) based on a double-antibody method. Intra- and interassay coefficients of variation were 6 and 9%, respectively. Concentrations of plasma non-esterified fatty acids (NEFA) were determined with a kit (NEFA C test, Wako Pure Chemical Industries Ltd., Japan). Concentrations of plasma lactate were determined as described by Taylor (1996). Volatile fatty acid (VFA) concentrations in the rumen and plasma were determined by gas chromatography (5890, Hewlett-Packard Co., United States) after steam distillation.

Calculations

Mean values with standard errors are given. The turnover rate of blood glucose was calculated using the equation described by Tserng & Kalhan (1983).

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

where R is the turnover rate of blood glucose, I is the [$U\text{-}^{13}\text{C}$]glucose infusion rate, and E is the plasma glucose enrichments during steady states.

All data were analysed with the MIXED procedure of SAS (1996). The split-plot design was used to test for the effects of diet, environment, and their interaction. The main plot was diet, the subplot was environment and the interaction, and sheep was the block. For daily profiles, the repeated statement was used to analyse the effects of diet and environment interaction. The least squared means statement was used to compare the mean values in the thermoneutral environment and values on each day during cold exposure for each dietary treatment. Results were considered significant at

the $P < 0.05$ level. The tendency for the difference was at the $P < 0.1$ level.

RESULTS

The neutral detergent fibre, acid detergent fibre, and ether extract contents in the PL diet were lower than the OR diet (Table 1). Crude protein, crude ash, and gross energy contents were similar between the diets. Of the macrominerals, Ca content was higher for the PL diet than for the OR diet. Those for K, Mg, and P were similar between the diets. Dry matter intake (DMI) was numerically lower for the PL diet than for the OR diet, and was lower ($P = 0.03$) during cold exposure than in the thermoneutral environment (Table 2). Weight change did not differ between the diets, and the body weight tended to be reduced ($P = 0.07$) during cold exposure. In the thermoneutral environment, energy digestibility did not differ between the PL and OR diets (Table 3). In the prefeeding period, concentrations of total VFA, acetate, and *n*-butyrate in the rumen did not differ between the diets. Rumen concentrations of propionate ($P = 0.01$) and *n*-valerate ($P = 0.05$) were higher and those of *i*-valerate were lower ($P = 0.04$) for the PL diet than for the OR diet.

The concentrations of plasma glucose, lactate, insulin, acetate, and propionate were similar between the diets in the thermoneutral environment, whereas plasma NEFA concentrations tended to be lower for the PL diet than for the OR diet (Fig. 1, 2). For the OR diet, plasma glucose concentrations increased ($P < 0.05$) on the fourth day of cold exposure, but changes for the PL diet were not significant. For both diets, plasma NEFA concentrations increased ($P < 0.05$) during cold exposure, whereas the concentrations of plasma lactate and

Table 1 Chemical components and macrominerals of plantain (PL) and orchard grass (OR) fed to sheep.

Item	PL	OR
Dry matter (g kg ⁻¹ fresh)	125	183
Chemical composition (g kg ⁻¹ dry matter)		
Crude protein	148	140
Neutral detergent fibre	316	498
Acid detergent fibre	214	260
Crude ash	128	107
Ether extract	18	32
Gross energy (kJ g ⁻¹ dry matter)	19.7	20.5
Macrominerals (g kg ⁻¹ dry matter)		
K	43	43
Ca	20	2.9
Mg	1.8	1.4
P	3.4	3.0

Table 2 Dry matter intake (DMI), weight change, and blood glucose turnover rate in sheep fed the plantain (PL) and orchard grass (OR) diets in the thermoneutral (20°C) and cold (0–4°C for 5 days) environments.

Item	PL		OR		SE	<i>P</i> -value		
	Thermoneutral	Cold	Thermoneutral	Cold		Diet	Environment	Interaction
No. of sheep	4	4	4	4				
DMI (g day ⁻¹)	995	888	1178	1130	141	0.37	0.03	0.32
Weight change (kg day ⁻¹)	0.1	-0.2	-0.1	-0.1	0.05	0.95	0.07	0.09
Net glucose turnover rate (mg kg ^{-0.75} of body weight min ⁻¹)	5.7	8.8	5.9	8.8	0.51	0.89	0.002	0.79

insulin remained unchanged. Concentrations of plasma total VFA, acetate, and propionate for the PL diet decreased ($P < 0.05$) only on the third day of cold exposure, but decreases in plasma VFA concentrations for the OR diet were not significant ($P < 0.1$). No significant interaction of diet and environment was detected in any of the blood metabolites and hormones determined.

For the isotope dilution experiment, plasma glucose concentrations and the infusion rate and plasma enrichments of [$U-^{13}C$]glucose were virtually constant during the latter half of [$U-^{13}C$]glucose infusion. The isotope enrichments of plasma glucose for four treatments are shown in Fig. 3. Net blood glucose turnover rate did not differ between the PL and OR diets in either environment (Table 2). The rate was higher ($P = 0.002$) during cold exposure than in the thermoneutral environment. The diet and environment interaction was not detected.

DISCUSSION

The relatively lower DMI for the PL diet than for the OR diet seemed to be due to the physical form or dry matter contents of the diets rather than to the palatability of the diet. On the contrary, Niezen et al. (1998) reported that plantain which had matured to the reproductive stage was not palatable to lambs and the lambs performed poorly. Because the PL diet was used at the bolting stage, the palatability may have changed with the growing stage. Cold exposure usually increases feed intake of sheep due to increased passage rates of digesta and enhanced energy expenditure (Kennedy et al. 1976; Tsuda et al. 1984). However, DMI was reduced during cold exposure. This may be partly related to the ability

of feed consumption, form and temperature of fresh diets, environmental temperature, or duration of cold exposure. The tendency of reduced body weight during cold exposure was also observed. However, careful attention should be paid to interpreting the result because small numbers of animals were used, the duration of cold exposure was short, and the possibility could not be excluded that the changes could simply be a reflection of reduction in gut fill during cold exposure.

In the thermoneutral environment of the major VFA, acetate concentrations in the rumen were similar between the diets, whereas rumen propionate concentrations were higher ($P = 0.01$) for the PL diet than for the OR diet. Diurnal changes in rumen VFA concentrations are mainly related to feeding (Leedle et al. 1986; Peters et al. 1990). Therefore, the collection of rumen fluids throughout the feeding period would have given more detailed information than could be obtained from the prefeeding period in this experiment. In a preliminary experiment using a fistulated sheep, postprandial concentrations of propionate in the rumen increased as the PL:OR ratio of the diets was raised. The ratio of acetate to propionate in the rumen was low when cattle were fed high-concentrate diets (Sutton et al. 1986; Peters et al. 1990). Therefore, it seems that the PL diet results in a more concentrate-type fermentation than the OR diet. The enhanced propionate concentrations in the rumen for the PL diet were not reflected in blood propionate concentrations possibly because most of the propionate was removed by the rumen epithelium and the liver (Bergman 1990).

Cold exposure was reported to reduce the production rate of propionate in the rumen and

Table 3 Energy digestibility and total and individual volatile fatty acids (VFA) in the rumen of sheep fed the plantain (PL) and orchard grass (OR) diets in the thermoneutral environment (20°C). ^aThe prefeeding period.

Item	PL	OR	SE	<i>P</i> -value
No. of sheep	4	4		
Energy digestibility (%)	61	60	3.4	0.68
VFA in the rumen ^a (mmol litre ⁻¹)				
Total VFA	69	67	2.3	0.69
Acetate	44	47	1.7	0.38
Propionate	16	12	1.1	0.01
<i>i</i> -Butyrate	0.5	1.0	0.09	0.06
<i>n</i> -Butyrate	8	5	0.7	0.20
<i>i</i> -Valerate	0.5	1.3	0.13	0.04
<i>n</i> -Valerate	0.8	0.6	0.05	0.05

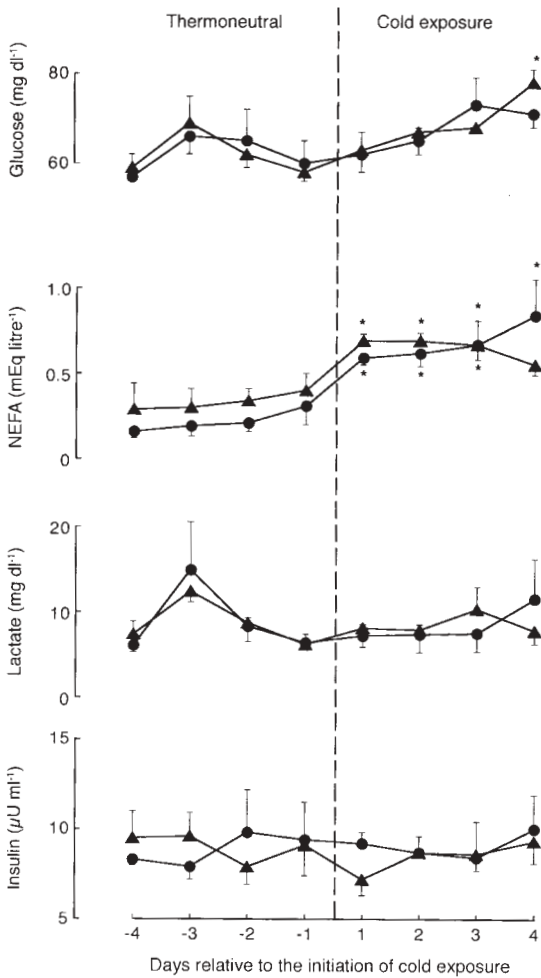


Fig. 1 Concentrations of plasma glucose, nonesterified fatty acids (NEFA), lactate, and insulin in sheep fed either the plantain diet (circles) or the orchard grass diet (triangles) and exposed to a thermoneutral (20°C) and cold (0–4°C) environment. Data expressed as means with standard errors in each treatment of four sheep. Asterisks indicate differences ($P < 0.05$) from the thermoneutral mean values for each treatment.

blood VFA concentrations in sheep (Tsuda et al. 1984; Sano et al. 1995). More acute cold exposure enhanced VFA absorption and hepatic propionate uptake in sheep (Thompson et al. 1978). These data might explain the decreases in plasma VFA, acetate, and propionate concentrations on the third day of cold exposure in the present experiment.

Neither diet nor cold exposure influenced basal concentrations of plasma insulin. However, possibilities that the insulin status of the sheep

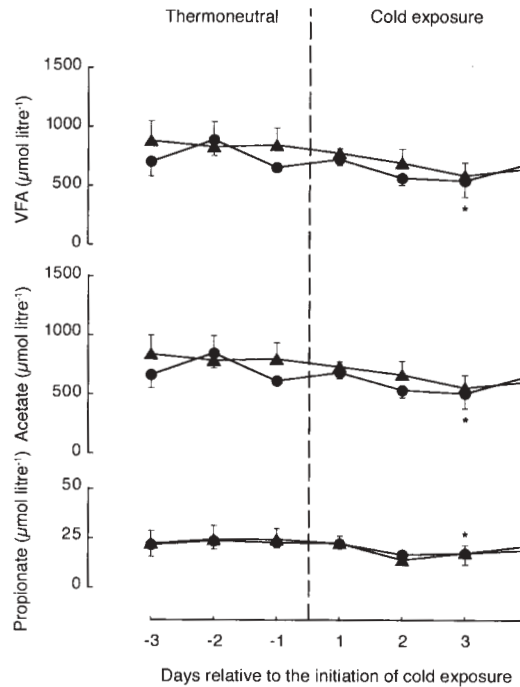


Fig. 2 Concentrations of plasma total volatile fatty acids (VFA), acetate, and propionate in sheep fed either the plantain diet (circles) or the orchard grass diet (triangles) and exposed to a thermoneutral (20°C) and cold (0–4°C) environment. Data expressed as means with standard errors in each treatment of four sheep. Asterisks indicate differences ($P < 0.05$) from the thermoneutral mean values for each treatment.

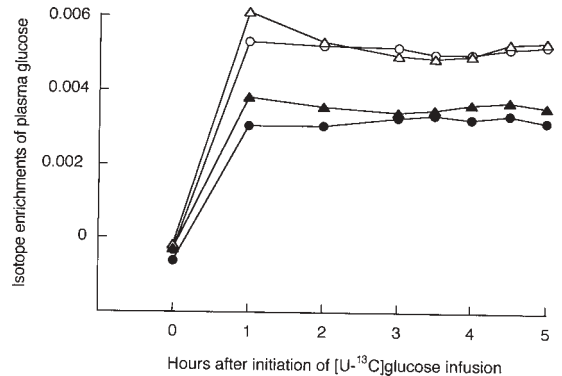


Fig. 3 Isotope enrichments of plasma glucose during the infusion of [U-¹³C]glucose (priming dose, 10 mg; infusion rate, 8 µg kg^{-0.75} of body weight min⁻¹). Data expressed as means in four sheep for four treatments (open circles, the plantain diet in the thermoneutral environment; closed circles, the plantain diet during cold exposure; open triangles, the orchard grass diet in the thermoneutral environment; closed triangles, the orchard grass diet during cold exposure).

differed could not be excluded because both factors influenced insulin responses to secretagogues and insulin action (Sasaki & Takahashi 1980; Sasaki et al. 1982; Weekes et al. 1983; Sutton et al. 1986; Sano et al. 1995, 1999).

Blood glucose turnover rate increased with increasing digestible energy intake (Weekes 1979). Digestible energy intake seemed to be lower for the PL diet than for the OR diet because DMI was 20% lower for the PL diet than for the OR diet, but not statistically significant, and digestible energy contents were similar between them. Moreover, net blood glucose turnover rates were determined between 2 and 4 h after the initiation of feeding to amplify any dietary effect, but the rates did not differ between the diets. In this regard, propionate is quantitatively the most important single precursor of glucose (Bergman 1990). Therefore, the difference in propionate availability from the rumen may partly explain the similar net glucose turnover rates for the PL and OR diets. Rodriguez et al. (1985), however, reported that in lactating goats propionate infusion into the rumen failed to influence percentage of glucose derived from propionate, amount of propionate converted to glucose, and glucose turnover.

Cold exposure enhanced ($P = 0.002$) net turnover rates of blood glucose in sheep as reported previously (Weekes et al. 1983; Tsuda et al. 1984; Sano et al. 1999). Weekes et al. (1983) reported that in sheep fed 1 kg of alfalfa pellets glucose irreversible loss rates were 1.4 times greater during cold exposure ($0 \pm 0.5^\circ\text{C}$) than in the thermoneutral environment and found that there were no consistent changes in irreversible loss between 7 and 20 days of cold exposure. Early et al. (1990) obtained the similar results in sheep exposed to $0-4^\circ\text{C}$ chronically (21–25 days). Takebayashi et al. (1998) determined blood glucose metabolism in sheep immediately after the initiation of cold exposure ($1 \pm 1^\circ\text{C}$) and found that rates of glucose production and utilisation increased to 1.7 times at 30 min after the initiation of cold exposure. Therefore, it seems that cold exposure enhances blood glucose kinetics without the clear duration effect.

No significant interaction between diet and environment was observed in relation to feed intake, weight change, net blood glucose turnover rate, and other measurements. This may suggest that the PL diet is comparable to the OR diet in both thermoneutral and cold environments. However, the possibility could not be excluded that cold exposure was a considerably strong stress for estimating the

dietary effect, as observed previously (Sano et al. 1999).

In conclusion, the forage herb PL has been shown to be a comparable forage diet to OR in respect of digestive and metabolic characteristics and blood glucose metabolism in sheep.

ACKNOWLEDGMENT

This study was in part supported by the Research Project for Activation of Joboji Town, Iwate Prefecture, Japan.

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