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Effects of Plantain (*Plantago lanceolata* L.) Herb and Heat Exposure on Plasma Glucose Metabolism in Sheep

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ABSTRACT : An experiment was conducted using a [6, 6-^{2}H]glucose isotope dilution method to determine the effects of plantain (*Plantago lanceolata* L.) on plasma glucose metabolism in sheep taken from a thermoneutral environment and exposed to a hot environment. The sheep were fed either mixed hay (MH) of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) at a 60:40 ratio or MH and plantain (PL) at a 9:1 ratio in a crossover design for each 23-day period. In both dietary treatments the metabolizable energy (ME) and crude protein intake were designed to be isoenergetic and isoproteinous at around maintenance level. The sheep were taken from a thermoneutral environment (20°C, 70% RH) and exposed to a hot environment (28-30°C, 70% RH) for 5 days. The isotope dilution method using a single injection of [6, 6-^{2}H]glucose was performed on the 18th day of the thermoneutral environment and on the 5th day of heat exposure. Plasma glucose pool size was numerically lower ($p = 0.26$) during heat exposure on both dietary treatments, and numerically higher ($p = 0.13$) on the MH diet irrespective of environmental temperature. Plasma NEFA concentration ($p = 0.01$) and glucose turnover rate ($p = 0.03$) were decreased during heat exposure, but remained similar between diets. It could be concluded that, although no positive impact of plantain on glucose metabolism was found under the present experimental conditions (plantain constituted only 10% of basal diet), plantain herb is an alternative to MH for rearing sheep in both thermoneutral and hot environments. (**Key Words** : Plantain Herb, Heat Exposure, Stable Isotope, Glucose Metabolism, Sheep)

INTRODUCTION

Heat exposure is a major constraint which reduces the productivity of ruminants through reduced feed intake, elevated body temperature, impaired prenatal function and prenatal development. Heat exposure also influences the concentrations of blood metabolites and modifies endocrine secretions (Bell et al., 1987; Bell et al., 1989; Achmadi et al., 1993; Itoh et al., 2001).

The use of antibiotic growth promoters in animal feed risks possible drug resistance in human pathogenic bacteria. As a result, feed manufacturers and animal producers are concentrating on natural herbs as an alternative to synthetic antibiotics. Plantain (*Plantago lanceolata* L.) is one of the perennial herbs having some bioactive compounds such as acteoside, aucubin, and catalpol (Ishiguro et al., 1982; Nishibe and Murai, 1995), which have anti-oxidative activity (Zhou and Zheng, 1991; Wang et al., 1996) and anti-inflammatory effects (Murai et al., 1995; Marchesan et al., 1998).

In ruminants dietary carbohydrates are fermented to volatile fatty acids, the major energy source, by microorganisms in the rumen. Therefore, little glucose is absorbed from the digestive tract and must be supplied through gluconeogenesis. Glucose metabolism is influenced by nutritional and physiological conditions (Evans and Buchanan-Smith, 1975; Buckley et al., 1982; Sano et al., 1983). Sano et al. (1983) also reported that glucose metabolism was reduced during heat exposure.

Therefore, it was expected that, due to the presence of bioactive components, plantain might influence the responses to glucose metabolism through reducing heat stress in ruminants. However, until now, to the best of our knowledge, the effect of plantain herb during heat exposure on intermediary metabolism of glucose in sheep has not been reported.

The present experiment was conducted to determine the effect of plantain on plasma glucose metabolism in sheep during heat exposure (30°C and 70% relative humidity (RH)), using an isotope dilution method with [6, 6-^{2}H]glucose.

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MATERIALS AND METHODS

Animals, diets, and heat exposure

Six crossbred (Corriedale×Suffolk) shorn sheep of both sexes (*Ovis aries* L.), aged around 2 years and with mean body weight (BW) of 30±2 kg were used. The sheep were housed in individual pens in a barn during the first 2-week adjustment period of the experiment. Two dietary treatments were used: (1) mixed hay (MH diet) of orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) (60:40, 1.78 kcal ME/g air dry matter (DM), 9.8% crude protein (CP)); (2) MH: plantain (PL diet, 9:1). The metabolizable energy (ME) and crude protein intakes were designed to maintain around maintenance level (NRC, 1985) on both dietary treatments. The animals were fed once daily at 14:00 h, and usually ate everything within 1 h, although the manger was untouched till next morning. Water was available *ad libitum*.

The experiment was performed using a crossover design with two 23-day periods in which either MH or PL diets were fed. Three sheep were fed the MH diet during the first period and then the PL diet during the second period, and the other three sheep were fed in the reverse order. On day 14, the animals were moved to metabolism cages in a controlled environment chamber at an air temperature of 20±1°C, 70% relative humidity (RH) and with lighting from 08:00 h to 22:00 h. An isotope dilution method using [6, 6-^{2}H]glucose was conducted on day 18 of each dietary period to determine plasma glucose metabolism in the thermoneutral environment (20±1°C). The environmental temperature was then elevated and maintained at 28-30°C, 70% RH with lighting from 08:00 h to 22:00 h for 5 days, and the same isotope dilution method was performed on the 5th day of heat exposure. The sheep were weighed at the start of the experiment, on day 14 of the experiment and after finishing each environmental treatment.

Two catheters, one for isotope infusion and another for blood sampling, were inserted into the left and right jugular veins on the morning of each isotope dilution procedure. Catheters were filled with sterile solution of trisodium citrate (0.13 mol/L). Blood sampling was performed without noticeable stress to the sheep. The handling of animals, including cannulation and blood sampling, was carried out according to the rules and regulations established by the Animal Care Committee of the Iwate University.

Experimental procedures

Rectal temperature was measured at 14:00 h for 3 days during both thermoneutral and heat exposure. At 12:00 h on the day of the isotope dilution method, 100 μmol of [6, 6-^{2}H]glucose (D-glucose, 6, 6-D_2, 99 atom% excess D, Isotec, A Matheson, USA Co., Miamisburg, USA) dissolved in 10 ml of saline solution (0.15 mol sodium chloride/L) was injected through the jugular infusion catheter. Blood samples (5 ml) were collected from the sampling catheter immediately before and at 20, 40, 60, 90 and 120 min after the isotope injection for the determination of plasma glucose concentration, pool size and turnover rate. Samples were transferred into heparinized centrifuge tubes and were chilled until centrifugation.

Analyses

Blood samples were centrifuged at 12,000×g for 10 min at 2°C (RS-18 IV, Tomy, Tokyo, Japan), and the plasma was stored at -30°C until further analysis. Isolation and derivatization of plasma glucose was performed by the procedure of Tserng and Kalhan (1983) and Wolfe (1984) with slight modification by Fujita et al. (2007). The isotopic abundance of the glucose derivative was determined with a gas chromatography mass spectrometry system (QP-2010, Shimadzu, Kyoto, Japan) with selected ion monitoring. Concentration of plasma glucose was enzymatically determined by the method of Huggett and Nixon (1957). Concentration of plasma nonesterified fatty acids (NEFA) was enzymatically determined as described previously (Fujita et al., 2006) with a kit (NEFA C test, Wako Pure Chemicals, Osaka, Japan).

Calculations

Plasma glucose pool size and turnover rate were calculated according to a single-pool analysis (Wolfe, 1984). The sampled pool represents the blood, which is in rapid equilibrium with the interstitial fluid (Schmidt and Keith, 1983).

Statistical analysis

All data were analyzed with the MIXED procedure of SAS (1996). The lsmeans statement was used to test the effects of period, diet, environment and interaction of diet and environment. The random effect was sheep. Results were considered significant at the $p<0.05$ level, and a tendency was defined as $0.05\leq p<0.10$. The repeated statement and the Tukey-Kramer adjustment were used for the time course of changes and the significance level was $p<0.05$ and $p<0.10$, respectively.

RESULTS

Daily profiles

Body weight of animals on MH and PL diets remained unchanged in both environments. Rectal temperature was higher ($p = 0.007$) during heat exposure on both diets and there was a trend to higher ($p = 0.09$) on MH diet than PL

Table 1. Effects of plantain supplementation and heat exposure on rectal temperature and plasma non-esterified fatty acid (NEFA) concentration in sheep

	MH*		PL		SEM	Significance		
	TN	Heat	TN	Heat		Diet	Env	Diet×Env
No. of sheep	6	6	6	6				
Rectal temperature (°C)	39.6	40.0	39.4	40.0	0.1	0.53	0.007	0.09
NEFA (mmol/L)	0.24	0.18	0.25	0.15	0.03	0.75	0.01	0.45

* MH = Orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) hay (60:40).
PL = Orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) hay mixture and plantain (9:1 ratio).
TN = Thermoneutral (20°C); Heat = Heat exposure (28-30°C); Env = Environment; Diet×Env = Diet and environment interaction.
SEM = Standard error of the mean.

Table 2. Effects of plantain supplementation and heat exposure on concentration, pool size and turnover rate of plasma glucose in sheep

	MH*		PL		SEM	Significance		
	TN	Heat	TN	Heat		Diet	Env	Diet×Env
No. of sheep	6	6	6	6				
Glucose concentration (mmol/L)**	3.4	3.4	3.5	3.2	0.03	0.47	0.13	0.20
Pool size (mmol/kg $BW^{0.75}$)	2.3	2.1	2.1	1.9	0.1	0.13	0.26	0.95
Turnover rate (mmol/kg $BW^{0.75}$/day)	37	32	40	33	2	0.52	0.03	0.92

* MH = Orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) hay (60:40).
PL = Orchardgrass (*Dactylis glomerata* L.) and reed canarygrass (*Phalaris arundinacea* L.) hay mixture and plantain (9:1 ratio).
TN = Thermoneutral (20°C); Heat = Heat exposure (28-30°C); Env = Environment; Diet×Env = Diet and environment interaction.
SEM = Standard error of the mean.
** Mean values of the plasma glucose concentrations during 120 min of each experiment.

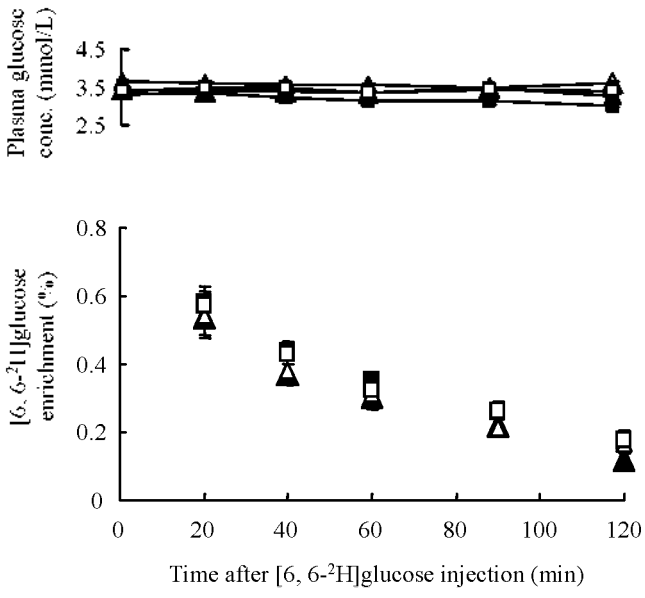

Figure 1. Time course of changes in plasma glucose concentrations and plasma [6, 6-^{2}H]glucose enrichments after a single injection of [6, 6-^{2}H]glucose in sheep fed the MH diet in thermoneutral and heat exposure (open triangle and square, respectively) and PL diet in thermoneutral and heat exposure (solid triangle and square, respectively).

diet (Table 1). Plasma NEFA concentration decreased during heat exposure ($p = 0.01$), and remained similar between diets.

Blood glucose metabolism

Plasma glucose concentrations remained constant during the 120 min period in each experiment (Figure 1). Plasma [6, 6-^{2}H]glucose enrichments decreased as a single-exponential function from 20 min to 120 min after [6, 6-^{2}H]glucose injection. Plasma glucose concentrations were unchanged with dietary and environmental treatments ($p = 0.47$ and $p = 0.13$, respectively, Table 2). Plasma glucose pool size was numerically lower ($p = 0.26$) during heat exposure in both dietary treatments, and numerically higher ($p = 0.13$) on MH diet irrespective of environmental temperature. Plasma glucose turnover rate decreased ($p = 0.03$) during heat exposure, but remained similar between diets ($p = 0.52$).

DISCUSSION

In the present study rectal temperature increased ($p = 0.007$) during heat exposure which agrees with previous findings (Achmadi et al., 1993; Itoh et al., 2001). Plasma NEFA concentrations decreased during heat exposure ($p = 0.01$), but an effect of plantain was not observed ($p = 0.74$). However, plasma NEFA concentration tended to be lower in sheep fed the plantain diet than for the orchardgrass diet (Sano et al., 2002). The inconsistency might be due to the small amount of plantain (10% of MH was replaced by plantain) used in the present experiment. In another study, Sano et al. (1983) reported that plasma NFFA concentrations decreased during heat exposure in sheep, which agrees with our findings. They also reported that concentration of plasma thyroxine decreased and respiration rate increased markedly during heat exposure. Therefore, considering the physiological responses and blood

constituents, sheep were assumed to be influenced by heat exposure.

The plasma glucose concentrations were unchanged ($p = 0.13$) during heat exposure and between diets ($p = 0.47$) in the present experiment. Basal glucose concentration was not significantly lower during heat (28°C) exposure (Itoh et al., 1998) in lactating dairy cows administered i.v. with glucose, arginine and butyrate, which agrees with the present findings. However, plasma glucose concentrations were lower ($p<0.01$) in sheep during heat exposure as mentioned previously (Achmadi et al., 1993). The contrast may partly be due to a difference of dietary composition as these authors fed a diet containing a large proportion of concentrate. It has been found that lower forage to concentrate ratios in diets increases the efficiency of energy utilization during heat exposure (Beede and Collier, 1986). It has also been reported that basal glucose concentration decreases during heat exposure (30°C) in ewes fed alfalfa hay cubes at maintenance level (Itoh et al., 2001). The inconsistency of these findings might be related to animal species, physiological and environmental conditions, feed composition and feeding regimen.

A number of experiments to determine glucose metabolism have been done using either the single injection or primed continuous infusion methods of glucose isotope dilution (Kronfeld and Simesen, 1961; Buckley et al., 1982; Sano et al., 2007). In the present study we used the single injection method of [6, 6-^{2}H]glucose dilution, because, unlike primed continuous infusion, it allows measurement of plasma glucose pool size in addition to glucose turnover rate. However, Buckley et al. (1982) applied both single injection of [6-^{3}H]glucose and the primed-continuous infusion of [U-^{13}C]glucose for determination of glucose metabolism in lactating and non-lactating goats, and reported that glucose irreversible loss rates were about 20% greater for the single injection method. However, the values of this parameter determined in the present experiment were comparable between treatments. In the present study, the numerical values of plasma glucose pool size were lower during heat exposure in both dietary treatments. In a previous study, plasma glucose pool size tended to decrease during heat (30°C) exposure in sheep (Sano et al., 1979) which agrees with the present findings. The mean values of the plasma glucose pool size in sheep, fed alfalfa hay cube and concentrate mixture twice daily, in a previous study (Sano et al., 1979) were slightly higher than the values found in the present experiment. This might be due to differences in the intake, feeding regime and frequency and composition of feed. However, plasma glucose pool size remained unchanged after feed restriction in lactating ewes (Gow et al., 1981), and glucose pool size was hardly influenced by energy and dry matter intake (Schmidt and Keith, 1983).

Blood glucose turnover rate decreased during heat exposure ($p = 0.03$) on both diets, although dietary intake was not influenced. Similar results were observed in sheep fed alfalfa hay cube and commercial concentrate (1:1) during heat exposure (30°C) (Sano et al., 1983), and in sheep exposed to 30°C and fed lucerne hay with chromium supplementation (Sano et al., 2000). Whole-body glucose kinetics are also influenced partially by endocrine hormones. Synthesis of thyroxine and triiodothyronine were reduced in lactating cows when exposed to heat (31.2°C) for 10 days (Magdub et al., 1982), and concentration of thyroxine in plasma was decreased in sheep during heat exposure (Sano et al., 1983). Triiodothyronine concentration is directly related to glucose utilization (Saunders et al., 1978), which is regulated by the activities of glycolytic enzymes (Lombardi et al., 2000). Whole-body glucose utilization in sheep and glucose utilization in adipose tissue of fat-fed rats were stimulated by epinephrine (Susini et al., 1979; Sano et al., 1996). Epinephrine secretion is expected to have been reduced during heat exposure, because plasma NEFA, which is mobilized by epinephrine, was reduced during heat exposure. Blood glucose turnover rate may be influenced by hormones as well as type of diet, energy intake, and physiological status (Evans and Buchanan-Smith, 1975; Weekes, 1979; Janes et al., 1985; Ortigues-Marty et al., 2003; Sano et al., 2007).

Few experiments have been conducted to study glucose metabolism in animals fed plantain herb, except for an experiment by Sano et al. (2002) in which glucose metabolism in sheep was studied using [U-^{13}C]glucose. Although not significant, the numerical values of glucose turnover rate were higher for the PL diet in both the thermoneutral and hot environments in the present experiment. This might partly be related to the availability of volatile fatty acids as the energy source. The concentration of propionate, a major glucose precursor, in the rumen was higher on the plantain diet than on the orchardgrass diet in sheep whereas acetate concentration decreased slightly (Sano et al., 2002).

It could be concluded that plasma glucose metabolism was influenced by heat exposure and remained similar between diets. No significant interaction between diet and environments was observed in relation to glucose turnover rate, suggesting that plantain herb is comparable to MH diet in both thermoneutral and hot environments. It was hypothesized that the bioactive components present in plantain could improve the metabolism but no positive impact was found under the present experimental conditions (only 10% of MH was replaced by plantain). Thus, further investigations need to be performed with an increasing amount of plantain in the basal diet.

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