Asian-Aust. J. Anim. Sci.
Vol. 20, No. 10 : 1575 - 1579
October 2007

www.ajas.info

Glucose Tolerance and Insulin Response to Intravenous Glucose Load in Sheep Fed on Germinated Sorghum Grain

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ABSTRACT : The glucose tolerance and pancreatic insulin secretion response to glucose in sheep fed on germinated sorghum grain were determined using an intravenous glucose load. Twelve male Thin Tail sheep (an Indonesian native sheep, 12 months old and 14.8 kg average body weight) were divided randomly into sorghum grain-based (S), germinated sorghum grain-based (G) and maize grain-based (C) diets. Sheep were maintained at the same daily intake levels of metabolizable energy and crude protein in the diets throughout the experimental period. After two months of the experimental conditions, each diet group was subjected to an intravenous glucose load experiment in which five doses of glucose (0, 10, 20, 40 and 80 mg/kg BW) were injected to estimate the rate of glucose removal from blood and the pancreatic insulin secretion response. For each sheep and each glucose load dose, the incremental blood serum glucose and insulin concentrations above pre-injection concentration were calculated as serum glucose and insulin response areas. At all glucose doses, sheep fed on S diet had a greater ($p<0.05$) glucose response area compared to those of sheep fed on G and C diets. Likewise at all glucose doses, the insulin response area was smaller ($p<0.05$) in sheep fed on S diet than in sheep fed on G and C diets. The glucose and insulin response areas in sheep fed on G and C diets differed slightly. It was concluded that the portion of maize grain in the ruminant ration could be substituted by germinated sorghum grain. (**Key Words :** Glucose, Insulin, Sorghum, Sheep)

INTRODUCTION

The oral glucose tolerance test has long been applied as a routine measurement of glucose utilization by body tissues in man. The test indicates the ability of body tissues to remove glucose from the blood. An intravenous glucose tolerance test, a modification of the oral glucose tolerance test, has been applied for quantifying glucose metabolism in ruminants (Sasaki et al., 1984; McCann et al., 1986; Waghorn et al., 1987; Cole et al., 1993). Insulin is known to be central to metabolic regulation in ruminants and, as in non-ruminants, plasma insulin is increased after feeding in ruminants (Sasaki et al., 1984; Sasaki and Watanabe, 1990). This reflects an augmented release of insulin by the pancreas (Bassett, 1978). The ruminant mode of digestion has been considered to modify the physiological stimuli for insulin secretion and to downgrade the importance of stimulating removal of an exogenous glucose load among the many actions of this hormone (Weekes, 1991).

The use of maize grain in the diet of ruminants is restricted by the high cost of feed. Sorghum grain has the potential to substitute a portion of maize grain in the ruminant diet because the energy content of sorghum grain is almost similar to that of maize grain. The quantity of sorghum grain production is dominant even throughout the dry season in the island of Java. Though the cost of a sorghum grain-based diet is lower than that of a maize grain-based diet, the use of sorghum grain is restricted by its tannin content. There have been some attempts to reduce the tannin content of sorghum grain and therefore enhance nutrient utilization (Reichert et al., 1980; Mitaru et al., 1985; Sumiati and Suci, 1996). The nature of the germination process for sorghum grain may reduce its tannin content, and may thereby increase its nutrient utilization. Therefore, it is postulated that germinated sorghum grain may increase glucose availability in ruminants because starch digestion in the small intestine may be stimulated. In the present study, the insulin secretory response to intravenous glucose loads and the rate of removal of injected glucose from blood were determined in sheep fed on sorghum-based (S), germinated sorghum-based (G) and maize-based (C) diets.

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Received July 6, 2006, Accepted November 14, 2006

Table 1. Ingredients and chemical composition of experimental diets (% of dry matter)

	Diet		
	Sorghum grain-based	Germinated sorghum grain-based	Maize grain-based
Ingredients[1]			
Elephant grass	40.0	40.0	40.0
Unprocessed sorghum grain	54.0	0.0	0.0
Germinated sorghum grain	0.0	59.0	0.0
Maize grain	0.0	0.0	54.0
Rice bran	5.0	0.0	5.0
Mineral premix[2]	1.0	1.0	1.0
Chemical composition[3]			
Crude protein (%)	9.9	9.5	9.6
Metabolizable energy[4] (Mcal/kg)	2.6	2.6	2.6
Tannin (%)	2.0	0.4	0.0

[1] Based on dry matter.
[2] Contained per 100 g mineral premix: 50 $CaCO_3$; 25 P; 0.35 Mn; 0.20 I; 0.10 K; 0.15 Cu; 22.00 Na; 0.80 Fe; 0.20 Zn; 0.15 Mg; 1.05 Cl.
[3] By analyses.
[4] Calculated metabolizable energy based on Hartadi et al. (1994).

MATERIALS AND METHODS

Diets and animals

The feed grade of sorghum grain was germinated for 48 h in a room, and then was sun-dried. The germination was done by spreading the sorghum grain over a water-moist cloth, which was maintained moist throughout the germination process. The sun-drying was ended when the moisture content of germinated sorghum grain reached 20%.

Twelve male 'Thin Tail', Indonesian native, sheep used in the present study were 12-mo old and their body weights averaged 14.8±1.9 kg. Sheep were allotted to the experimental treatments with diet as a fixed effect, divided randomly into S, G and C diet groups, and they were housed individually in metabolic cages in a room. Each diet was prepared to provide the same amounts of energy and protein intake on the basis of calculated metabolizable energy (ME) and crude protein contents, respectively (Table 1). Animals were fed daily at 130% of daily ME requirement for maintenance based on metabolic body size (NRC, 1985). Drinking water was available at all times except during the intravenous glucose load experiment.

Intravenous glucose load experiment

After two months of preconditioning on each dietary treatment, each diet group was subjected to an intravenous glucose load experiment. The intravenous glucose load was administered to each sheep after fasting overnight. An implanted jugular catheter was used for glucose injection and blood collection. In the intravenous glucose load experiment, sterile glucose (20% dextrose) was injected at doses of 0 (sterile saline), 10, 20, 40, and 80 mg/kg BW. The sequence of glucose dose in each sheep was assigned randomly with a 48 h interval between each glucose injection to avoid any carryover effect (Achmadi et al., 1993). The volume of injected glucose solution was 6 ml for all doses. Blood samples (5 ml) were collected at 5, 10, 15, 30, and 60 min after glucose injection and stored in ice-water until centrifugation for 10 min (4°C); harvested serum was used for glucose determination and insulin assay.

Serum glucose concentration was determined with a glucose assay kit (GLUCOSE liquicolor, Human Gesellschaft fur Biochemica und Diagnostica mbH, Taunusstein-Germany). Serum insulin concentration was determined by a radioimmunoassay method (Coat-A-Count® Insulin, Diagnostic Corporation, Los Angeles, USA); intra- and interassay CV were 5% and 11% respectively.

In the intravenous glucose load experiment, for each sheep and each dose of glucose, areas beneath the curve for serum glucose and insulin concentrations above pre-injection levels were calculated between 0 and 60 min after glucose injection. The effect of dietary treatment on feed intake and parameters derived from the glucose load experiment were tested by one-way ANOVA for a completely randomized design. The feed intake of each sheep was assessed using the average value of daily observation throughout the last 10 days of the two-month preconditioning period on each dietary treatment.

RESULTS

Dry matter, crude protein and metabolizable energy intakes of sheep throughout the experimental period are presented in Table 2. Although conditioned to different types of concentrate diet, sheep maintained dry matter and

Table 2. Means[1] of daily dry matter, crude protein, and metabolizable energy intakes of sheep throughout the experimental period

Items	Diet		
	Sorghum grain-based	Germinated sorghum grain-based	Maize grain-based
Intakes			
DM ($g/kg^{-0.75}/d$)	51.41±2.00	51.54±2.46	52.84±2.43
CP ($g/kg^{-0.75}/d$)	5.09±0.20	5.13±0.24	5.23±0.24
ME ($kcal/kg^{-0.75}/d$)	133.70±5.12	134.94±6.41	137.40±6.34

[1] Average values of 10 days observations in each sheep (mean±SD; n = 4).

Table 3. Parameter means[1] of the glucose load experiment in sheep

Items	Diet		
	Sorghum grain-based	Germinated sorghum grain-based	Maize grain- based
Basal glucose (mg/dl)	40.2±1.8[a]	43.4±2.0[b]	46.2±2.2[b]
Basal insulin (μU/ml)	11.3±1.2[a]	17.2±1.4[b]	20.8±1.7[b]
Glucose area (mg/dl/60 min)[2]			
0	0.0±0.0	0.0±0.0	0.0±0.0
10	184.7±29.7[a]	96.6±14.8[b]	90.8±25.0[b]
20	243.5±17.2[a]	124.0±17.5[b]	96.8±29.9[b]
40	469.7±8.9[a]	247.0±5.0[b]	236.9±12.0[b]
80	994.1±46.5[a]	539.9±36.3[b]	527.7±17.8[b]
Insulin area (μU/ml/60 min)[3]			
0	0.0±0.0	0.0±0.0	0.0±0.0
10	77.9±10.4[a]	146.3±4.4[b]	162.0±12.4[c]
20	110.4±11.3[a]	187.7±14.2[b]	207.8±10.3[c]
40	195.4±9.5[a]	345.3±4.4[b]	385.6±12.6[c]
80	407.0±8.7[a]	740.9±5.6[b]	818.6±11.2[c]

[1] Values are means of four sheep±SD.

[2] The serum glucose area beneath the curve for serum glucose concentrations above pre-injection concentration was calculated between 0 and 60 min after glucose injection at doses of 0, 10, 20, 40 and 80 mg/kg BW.

[3] The serum insulin area beneath the curve for serum insulin concentrations above pre-injection concentration was calculated between 0 and 60 min after glucose injection at doses of 0, 10, 20, 40 and 80 mg/kg BW.

[a, b, c] Means within a row with different superscripts letter are significantly different: $p<0.05$.

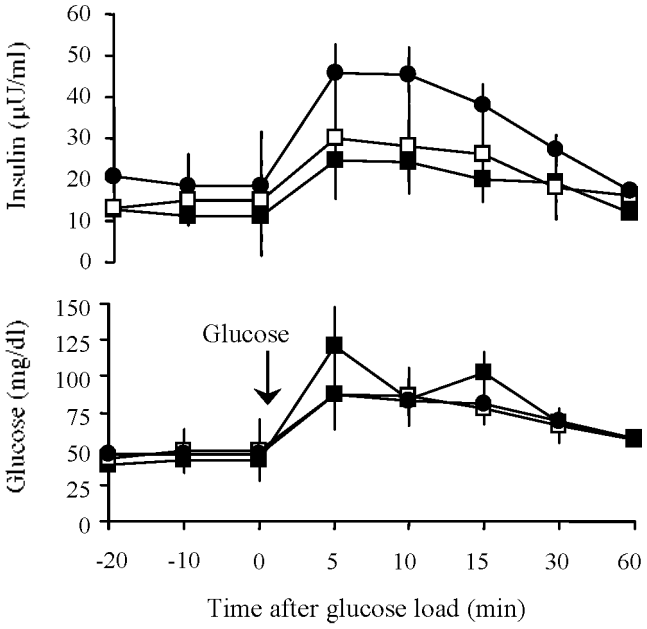

Figure 1. The serum glucose and insulin concentrations of sheep fed on sorghum grain-based (■), germinated sorghum-based (□), and maize grain-based (●) diets throughout the glucose load experiment. Each group received a glucose dose of 80 mg/kg BW. Values are means of four sheep with SD.

crude protein intakes to meet 130% of their daily metabolizable energy requirement for maintenance based on metabolic body size (NRC. 1985).

The dietary treatment affected basal serum glucose and insulin concentrations of sheep. The S diet lowered ($p<0.05$) basal glucose concentration of sheep compared to that on G and C diets (Table 3). Likewise, basal serum insulin concentration of sheep was lowered ($p<0.05$) by S compared to that on G and C diets. Though there was no significant difference between the effect of G and C dietary treatments on basal glucose and insulin concentrations of sheep. the C diet tended to increase basal glucose and insulin concentrations of sheep (Table 3).

Serum glucose concentrations of sheep rose abruptly following the intravenous glucose injection (Figure 1). The glucose area of sheep fed on the G diet tended to be higher than that of sheep fed on the C diet. but there was no significant difference between the values. The S diet increased ($p<0.05$) glucose area compared to that of the G and C diets. The magnitude of the glucose area was dose-dependent in all group of sheep. Mean glucose area plotted against glucose dose gave correlation equations of: $y = 12.1x+16.3$ ($r^2 = 0.99$); $y = 6.6x+4.3$ ($r^2 = 0.99$) and $y = 9.5x-4.3$ ($r^2 = 0.98$) for S. G and C diet groups. respectively.

Intravenous glucose injection caused an abrupt increase in serum insulin concentrations of sheep (Figure 1). Table 3 shows that the insulin area in sheep fed on the G and C diets differed slightly. The S diet decreased ($p<0.05$) insulin area compared to that on G and C diets. The magnitude of insulin area was glucose dose-dependent in all group of sheep. Mean insulin area plotted against glucose dose gave correlation equations of: $y = 4.9x+10.8$ ($r^2 = 0.99$); $y = 8.9x+16.3$ ($r^2 = 0.99$) and $y = 9.9x+18.8$ ($r^2 = 0.99$) for S, G and C diet groups. respectively.

DISCUSSION

Sheep fed on the S diet showed a greater glucose area

than that of sheep fed on G and C diets (Table 3). This indicated that sheep fed on the S diet had a greater volume of distribution of glucose and a slower removal rate of glucose from blood compared to sheep fed on G and C diets. The G and C diets may have provided greater glucose absorption compared to the S diet, though glucose absorption in the small intestine of sheep was not determined in the present experiment. Presumably, the germinated sorghum grain increased glucose availability because starch digestion in the small intestine may be stimulated. Janes et al. (1985) reported that processed maize grain provides an exogenous glucose which in turn improves metabolic clearance rate of glucose in sheep, compared to that of unprocessed maize grain.

Sasaki et al. (1984) confirmed that when exogenous glucose is administered, the rate of glucose disposal may relate to alterations in peripheral glucose utilization as well as to changes in the rate of hepatic glucose production. Sheep normally rely on continuous hepatic gluconeogenesis to meet their glucose requirement (Weekes, 1991). Very little glucose is absorbed from the small intestine in adult sheep fed on roughage diets, the majority of basal glucose flux being provided by hepatic gluconeogenesis which is assumed to be greater when sheep are fed concentrate diets (Achmadi et al., 1993).

Sheep fed on the G diet showed a greater serum insulin area ($p<0.05$) compared to that of sheep fed the S diet (Table 3). The improved insulin secretion response of sheep when fed the G diet was correlated with the enhanced glucose utilization. It has been reported that plasma insulin levels are not so clearly increased in response to feeding when animals are fed on a roughage diet compared with animals fed on concentrate diets (Trenkle, 1978; Cole et al., 1993). An increase in non-protein energy intake may enhance insulin sensitivity for glucose metabolism in adult goats (Fujita et al., 2006). However, tissue responsiveness and sensitivity to insulin may not be changed when goats were fed on dietary sucrose or dietary starch (Fujita et al., 2007). Although tissue responsiveness and sensitivity to insulin in intact sheep were not determined in this experiment.

The action of insulin to increase the rate of glucose disposal via suppression of hepatic glucose output and stimulation of hepatic glucose uptake seems to be a minor route of glucose disposal in sheep, since the suppression of hepatic glucose production by insulin has been reported to be relatively insensitive in ruminants (Weekes, 1983; Sasaki and Watanabe, 1990). However, elevated insulin concentration in plasma diminished hepatic uptake of glucose precursor (Brockman et al., 1986) and depressed hepatic glucose production (Bassett, 1975).

The use of glucose load could be considered as an exogenous stimulant for insulin release in sheep when simultaneously evaluating the rate of glucose metabolism. In this experiment, the removal rates of glucose from blood and insulin secretion responses to glucose load were dose-dependent in all sheep (Table 3). Although blood glucose concentration is usually considered to be the principal metabolic substrate regulating insulin release by the β cells of most animal species, the plasma glucose level itself may not be a major determinant of insulin secretion in sheep under physiological conditions (Bassett, 1975). Other metabolites, such as volatile fatty acids, have been suggested to be effective stimulants of insulin secretion in ruminants (Sasaki and Takahashi, 1980; Sasaki et al., 1984) in which volatile fatty acids are produced as end-products of rumen fermentation. However, glucose has been shown to be a direct stimulant for insulin release by perfused fragments of sheep pancreas (Sasaki et al., 1984).

CONCLUSION

The glucose tolerance and the insulin secretion response to intravenous glucose load of sheep fed germinated sorghum grain diet was similar to that of sheep fed a maize grain diet. However, the meat or milk products of germinated sorghum grain-fed ruminant remains to be elucidated before establishing the portion of maize grain in the ruminant ration that could be substituted by germinated sorghum grain.

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