Glucose Metabolism in Sheep Fed Grass Supplemented with *Gliricidia Sepium*

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ABSTRACT

The limiting factor on improving ruminant production for most of the available feed in developing countries are low in quality. Therefore high fibre diet must be supplemented by high nutritive feed such as leguminous trees that much available in those regions. *Gliricidia sepium* was one of very potential candidates. Glucose as a major energy source in fed animals required precursor in form of propionat and amino acids from diet. Those precursors might be supplied by these legume leaves. The aim of this research was to investigate the glucose metabolism in the sheep fed grass supplemented by *Gliricidia sepium*. Fifteen sheeps (18 months old) were used in the experiment. These are were divided into three groups that fed by experimental diet of Mitchell grass (MG group), Gliricidia (GS group), and MG supplemented with GS (MGGS group). D-[U-^{14}C]glucose infusate was infused continuously through the left jugular venous catheter of each animal to measure glucose metabolism in those sheeps. The measurements were done on feed utilisation and glucose metabolism. The results indicated that there was an improvement in efficiency of feed utilisation in the MGGS group as reflected by lower feed conversion ratio by the group. Plasma glucose concentration profile per unit of OM intake were similar for GS and MGGS groups, but higher than that in the MG group ($P<0.01$). Glucose entry rate (GER) increased in MG group through GS to the MGGS group, while N retention accordingly was increased. It can be concluded that the utilization of GS by the ruminant animal could be improved by feeding it with a low quality feed at a ratio of 40:60 (GS:Low quality feed) to achieve an NI:DOMI ratio of 0.03 - 0.04. This improvement would be manifested in increasing DOMI, with subsequent increase in GER or net protein deposition as might be expressed in positive N retention.

INTRODUCTION

Ruminant animals play a very important role in the lives of small-holder farmers in many countries. Small-holder farmers play a very important role in the economy of developing countries such as Indonesia, with a human population of approximately 235 million, obtains 34% of its total national meat production from ruminant animals. The small-holder farmers of that country supply about 89% of this meat [1].

Poor nutrition is the most important limiting factor to ruminant animal productivity in small-holder farming systems in developing countries. Most of the feeds available in such systems are high in lignified fibre and low in protein content. These feed characteristics become particularly prominent in the dry season, during which time animals grow very slowly [2] or lose live weight (LW). In this region, ruminants are mainly fed on crop residues generally receiving only 62% of their crude protein (CP) requirements [3]

The introduction of the shrub legume *Leucaena leucocephala* (Leucaena) had been quite successful in improving the quality of diets fed to ruminant animals [4]. However, this shrub legume is highly susceptible to psyllids (*Heteropsylla cubana*). It was important, therefore, to search for suitable shrub legumes that would be similar (or better) in nutritive value to Leucaena but would be resistant to psyllids. Such shrub legumes could be used as substitutes for Leucaena or to

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complement the role of Leucaena in ruminant animal feeding systems in small-holder farms.

More than 200 species of leguminous shrubs/trees have been tried as feeds for ruminant animals [5]. The most commonly used species come from the genera *Acacia, Albizia, Calliandra, Desmanthus, Desmodium, Gliricidia, Prosopis* and *Sesbania*. Among these, the shrub legumes *Gliricidia sepium* (Gliricidia) appear to show promising potential as feed supplement due to its high nutritive value, for conditions in Indonesia. The plants are fairly resistant to insect pests, and potentially have nutritive values that could match that of Leucaena [5-7]. The Gliricidia in particular, seem to be able to adapt well to the dry conditions that normally are experienced in the long dry season in the tropics.

To be considered seriously as animal feeds, shrub legume plants should have both desirable agronomic characteristics and high nutritive values [8,9]. However, their nutritive values in relation to ruminant animal feeding are needed to be defined. The value of a feed for animal productivity would be determined by the amount of the feed that an animal can eat voluntarily and the types of nutrient absorbed from the digestive tract of the animal fed the particular feed. In relation to growth, for example, it would be the amounts glucose made available to animals eating such shrub legumes that would be of particular interest. Quantitative information on the availability of these nutrients would be particularly useful when feeds are being considered as supplements to other basal feeds.

Glucose is a major energy source for ruminant animals that can be used for energy production [10]. Since glucose absorbed from the gastrointestinal tract of these animals is relatively small, most of the glucose is supplied from gluconeogenesis. In fed ruminant animals, the main precursors for gluconeogenesis are propionate and amino acids [11,12]. An increase in the rate of gluconeogenesis therefore might be expected when large amounts of dietary propionate and/or amino acids are made available to these animals as indicated by dietary nitrogen intake retained. Under conditions in which the ratio of absorbed dietary amino acids to energy (or essential amino acid to amino acid) is far in excess of that which is optimal for protein synthesis, the amino acids are likely to act as precursors for gluconeogenesis. The leaves of leguminous tree contained some glucogenic amino acids [13].

The result presented in this paper is a part of a seri of large experiment that determine the utilization of tropical shrub legumes for ruminant animals. The objective of the study presented in this paper was to determine the energy provided by *Gliricidia sepium* in form of glucose.

EXPERIMENTAL METHODS

Animals

The experiment used 15 head sheep (18 months old), which were randomely divided into three equal groups, evenly matched for LW following the Randomised Block Design [14]. The animals were kept in individual metabolism cages (55 cm x 120 cm per cage). Each animal has free acces to the experimental diet and clean drinking watter. Feed treatments offered to each group were Mitchell grass (*Astreblasp*) hay (MG group), Gliricidia (GS group), and MG supplemented with GS (MGGS group). The initial LW of animals was 27.6 ± 1.72 kg (MG group); 27.1 ± 2.26 kg (GS group) and 27.5 ± 1.56 kg (MGGS group).

The experiment was conducted with the Experimentation Ethics Approval No A635_01 in accordance with the *Guidelines for Housing and Care of Laboratory Animals* issued by the Experimentation Ethics Review Committee of James Cook University (JCU) at 2001.

The experiment was undertaken in the temperature-controlled room (temperature 22°C and humidity 74%).

Feed and feeding

The diets offered were designed to meet the maintenance metabolized energy (ME) requirement of the animals. The ME required for maintenance by animals used in the current experiment was estimated using the equation ME = 1.2 + 0.13 LW [15]. The ME content of GS and MG hay was 8.0 MJ/kg DM and 6.1 MJ/kg DM, respectively [13].

The GS leaves offered to the MGGS animals contributed 37% of the total DM of the daily ration of these animals. The inclusion of this proportion of GS was calculated to result in a dietary NI:DOMI ratio of 0.04 provided the relevant data on MG hay to provide nutrients balance required for rumen microbes activities [16].

Dry matter offered to the animals in the MG and GS groups was 815g and 578g/ day, respectively, and 254g of MG and 456g of GS per day for animals in the MGGS group, in order to supply the amount of energy metabolised required for maintenance. The ration for each animal was

divided into 12 equal portions. The five portions were given at 2-hourly interval from 0700 h to 1500 h. The rest of 7 portions were given to the animals at 1700 h. This feeding regimen was designed to provide a continuous flow of amino acids from the rumen into the duodenum, which should ensure a net amino acid absorption from the intestine [17].

During the glucose metabolism measurement, all animals received the same amount of DM (250 g) of their respective experimental diets. The amount offered to each animal was estimated to be one that the animal could consume within one hour.

Procedures

The experiment consisted of a 14-day period of adaptation to feeds and an 8-day measurement period. The measurement period consisted of seven days for feed utilisation determination and a day for observations on glucose metabolism. During measurement period, the feed intake and digestibility and glucose metabolism were observed. Sample of feed offered, total feed residues, total faeces excreted by the animal were collected each day. Then the sub-samples were taken from these, oven drying and grinding for DM and OM determination.

Catheterisation

Chronic in dwelling catheters was installed in the jugular veins, a femoral artery and a lateral saphenous vein of each sheep [17]. A polyethylene catheter (0.86 mm ID x 1.27 mm OD; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) was installed under local anaesthetic (Lignocaine 20; Troy Laboratories Pty. Limited, NSW Australia) in the femoral artery of the right hind limb of each sheep [17].

D-[U-^{14}C]glucose

In the last day of measurement period, the D-[U-^{14}C]glucose infusate (ordered from Amersham, Amersham International Inc., Buckinghamshire, England with the Purity of these was stated by Amersham to be 98.3%) was infused continuously through the left jugular venous catheter of each animal at the rate of 0.24 mL/min for six hours. From previous experience in the nutrition and fisiology Laboratory[18], it was assumed that plasma glucose specific radioactivity (SRA) would reach a plateau by four hours after the commencement of the infusion.

A 10 mL blood sample was withdrawn simultaneously from the femoral artery and from the lateral saphenous vein of each animal before the infusion of glucose tracer started. Subsequent pairs of 10 mL arterial and venous blood samples were taken, at 20-minute intervals, during the last two hours of tracer infusion.

Glucose concentration profile

To establish the patterns of plasma glucose concentration in sheep fed the experimental diets, blood samples were collected at half-hourly intervals from the right jugular vein catheter of each experimental animal just before it received its feed and for six hours immediately after feeding.

Five mL of blood were collected at each sampling time from each animal. The glucose concentration in the sample was analysed immediately using a Glucometer (ESPRIT, Bayer Corporation,USA). The remaining blood was centrifuged at 3000 g for 20 minutes, and the plasma was transferred into a 5 mL vial for further glucose analysis using the Auto-analyser.

Infusate radioactivity determination

The radioactivity of infusate D-[U-^{14}C] glucose was determined by counting 5µL of the infusate in 10 mL of Biodegradable Counting Scintillant (BCS) (Amersham Corporation, USA). The efficiencies for the counting of [^{14}C] was 82 %.

Glucose radioactivity and concentration

Blood glucose SRA was determined using the Glucose Penta Acetat (GPA) method [19]. Glucose concentrations in blood samples were determined using the Technicon Colorimetric Methods (Technicon Autoanalyser II, Germany).

Calculations feed intake and digestibility

Dry matter intake (DMI) can be derived from the equation (1) [19]:

$$DMI = DMO - DMF \quad (1)$$

DMI is the amount (g) of dry matter intake.

DMO is the amount (g) of dry matter offered.
DMF is the amount (g) of dry matter residual.

Dry matter digestibility (DMD) was calculated from the following equation (2):

$$DMD = \frac{DMI - DMF}{DMI} \times 100 \quad (2)$$

Nitrogen retained by an animal was calculated using the following equation[19]:

$$NR = NI - NF - NU \quad (3)$$

NR is the amount (g) of nitrogen retained.
NI is the amount (g) of nitrogen consumed by the animal.
NF is the amount (g) of nitrogen present in the faeces.
NU is the amount (g) of nitrogen present in the urine

Glucose entry rate was calculated using the equation (4) [17]:

$$GER = \frac{IR_{glu}}{Glu_{SRA}} \quad (4)$$

GER is the glucose entry rate (mg glucose/min)
IR_{glu} is the rate (MBq/min) of D-[U-^{14}C]glcucose infusion
Glu_{SRA} is the mean (MBq/mg glucose) plateau of venous glucose specific radioactivity (SRA_{gl})

The SRA is derived from the equation (5):

$$SRA_{gl} = \frac{DPM}{GPA} \times \frac{Glu_{carrier} + [glu]}{[glu]} \times \frac{Mwt_{GPA}}{Mwt_{glu}} \quad (5)$$

SRA_{gl} is the specific radioactivity (MBq/mg) of glucose
GPA is the amount (mg) of glucose pentaacetate formed
DPM is the amount (MBq/mg) of [^{14}C] radioactivity in the vial
[glu] is the concentration (mg/mL) of glucose in the blood plasma.
Mwt_{GPA} is the molecular weight (mg) of GPA
$Mwt_{glu}$is the molecular weight (mg) of glucose
Glu_{carier} is the amount (mg) of glucose added as a carrier

Statistical analysis

All raw data were tabulated using Microsoft®Excel (Microsoft Corporation, USA) and analysed using SPSS (SPSS Inc., USA). Graphs were produced using Microsoft®Excel (Microsoft Corporation, USA)

Data were analysed using ANOVA for the randomised block design [14]. Where significant effects of treatments were observed, differences among mean values were examined using Tukey's test [21].

RESULTS AND DISCUSSION

Feed utilisation and N balance

Protein contents of MG hay and GS was 3.7% and 17.1%, respectively. The data on feed utilisation and N balance in sheep fed the experimental diets are presented in Table 1.

Table 1. Mean* values of live weight (LW) and feed during the 8-day measurement period.

Variables	Treatments MG	GS	GGS	±SE
LW (kg)	27.6	27.1	27.9	2.6
LWG(g)	-90^{c}	28^{b}	80^{a}	53
DMI (g/d)	577^{b}	608^{b}	746^{a}	60
OMI (g/d)	511	69	12	71
DMI (g/kgLW$^{0.75}$)	50^{b}	53^{b}	62^{a}	3.4
OMI (g/kgLW$^{0.75}$)	44^{b}	49^{ab}	56^{a}	3.1
DMD (%)	44^{c}	55^{a}	49^{b}	1.4
OMD (%)	52^{c}	59^{a}	56^{b}	0.8
N intake (g/d)	3.4^{c}	15.7^{a}	10^{b}	0.8
N retained (g/d)	-0.12^{c}	0.80^{b}	1.84^{a}	0.7
N retained (%NI)	0^{c}	5.1^{b}	18.4^{a}	5.0
NI : DOMI	0.013^{c}	0.047^{a}	0.03^{b}	0.33
FCratio (gDMI/g LWG)	-	22^{b}	9^{a}	8.9

* Within rows, mean values with different superscript differ significantly (P< 0.05)

Note : LW = live weight; LWG = Live Weight Gain; DMI = intake of dry matter; OMI = organic matter, DMD and OMD = apparent digestibility of DM and OM; N = nitrogen; , NI:DOMI = ratio of N to digestible organic matter intake (DOMI); FC = feed conversion ratio; MG = Mitchell grass hay; GS = Gliricidia; MGGS = MG supplemented with GS.

Sheep in the MG and GS groups consumed similar amounts of DM but they consumed significantly less DM than the sheep in the MGGS group. The apparent DM and OM digestibility values of the MG hay plus GS were between the values observed for MG or GS only, with the lowest values recorded for the hay diet.

Sheep fed GS only had the highest N intake, followed by the animals in the MGGS and MG groups.The animals fed MG had a negative mean N balance, while those fed GS and the MG hay plus GS had positive N balance values. The amount of N intake retained in animals in the MGGS group was significantly higher than that in animals in the GS group.

Animals fed the MG lost LW during the observation period, while animals fed GS and MG hay plus GS gained LW. The gain in LW was significantly higher in animals in the MGGS group.

The feed conversion ratio was lower in the MGGS sheep than in the GS animals. The value of NI:DOMI ratio for the MGGS group was between the values recorded for the other two groups; the highest being observed in the GS group.

It is clear that the loss in LW of animals in the MG group was due to the low DM digestibility of the hay and also to its low CP content (3.7% of DM). The MG hay with low CP content (4-5%) observed has low digestibillity value (48%)[16]. Adding GS to MG hay, as in the MGGS group, resulted in increases in both intake and digestibility by 29% and 11% respectively. Observation on the addition of *Desmanthus spp* to Mitchell grass at 25% of DM intake improved digestibility by 5.6% [22]. The low quality grass used in the current experiment would be typical of the low quality roughage diets that are used in many of the developing countries.

The improvement in feed intake and digestibility of hay due to the addition of GS, most probably was due to the shift in NI:DOMI ratio of 0.013 in MG hay, to 0.03 in the MG hay plus GS diet. Balance between dietary energy and protein available in the diet that expressed as the ratio of NI:DOMI would facilitates the feed degradation in the rumen thus improving the synchronization between energy and protein in the rumen that support optimal rumen microbial protein synthesis [23]. Data obtained in the current study would suggest that further improvement might be obtained in the MG hay plus GS diet if the grass and shrub legumes components of the diet were to be fed at different times. A correlation between feed quality and microbial protein synthesis has been indicated[24]. The highest microbial protein synthesis was corresponding to the increasing of concentrate levels in the ration.

The improvement in efficiency of feed utilisation in the MGGS animals was reflected in the lower feed conversion ratio by the group. The MGGS group consumed a mean of 9 g of DM per unit of LWG compared with 22 g DM consumed per unit of LWG in the GS animals. The improved efficiency observed most likely was due to the better nutrient balance provided by the carbohydrate in the MG hay.

Glucose metabolism

Data on glucose metabolism in the whole body of sheep fed the experimental diets are shown in Table 2.

Dietary treatments did not significantly influence plasma glucose concentration and glucose entry rate in the whole body of animals. The profile of plasma glucose concentration in sheep

and Fig. 2. The profiles of plasma glucose concentration per unit of OM intake were similar in sheep in the GS and MGGS groups. The values for these groups were higher than that in the MG sheep (Fig. 1) ($P<0.01$). However, when the glucose concentrations were expressed per unit of OM intake digested, the differences in profiles among the treatment groups were negligible. The concentrations, generally, increased sharply within two hours after feeding time and remained at a plateau till the end of observation at six hours after feeding (Fig. 2). When feed restriction was applied there was a relationship between intake and glucose plasma concentration. Glucose plasma concentration decreased as intake was decreased [25].

Table 2. Mean values of plasma glucose concentration and glucose entry rate (GER) liberated from skeletal muscle of sheep fed Mitchell grass hay (MG), Gliricidia (GS) and MG supplemented with GS (MGGS) diets during measurement period.

	Treatments			
Variables	**MG**	**GS**	**MGGS**	**± SE**
Plasma glucose (mM)	4.82	5.02	5.28	0.29
GER (mmol/h)	17	22	23	3
(mmol/h/kgLW)	0.63	0.81	0.81	0.10
(per g DOMI)	0.28	0.29	0.30	0.04

* With assumption that skeletal muscle is 0.25 LW.

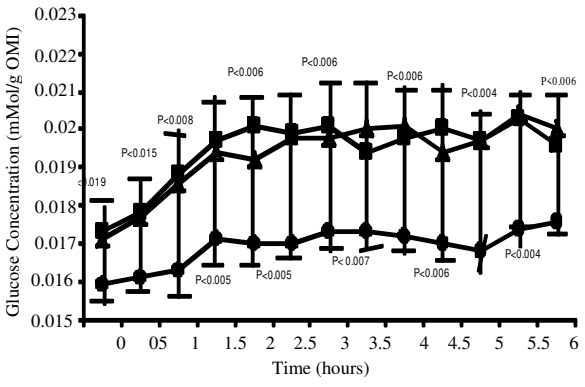

Fig. 1. The concentration of plasma glucose per unit of organic matter intake (OMI)) in sheep fed 250 g dry matter of Mitchell grass hay (■) (SEM ± 0.0015) or Gliricidia (▲)

(SEM ± 0.0018) or hay supplemented with Gliricidia (●) (SEM ± 0.0012).

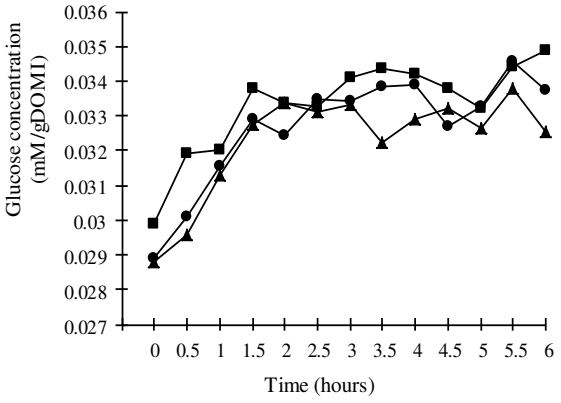

Fig. 2. The concentration of plasma glucose per unit of digestible organic matter intake (DOMI) in sheep fed 250 g dry matter of Mitchell grass hay (■) (SEM ± 0.0014) or Gliricidia (▲) (SEM ± 0.0012) or hay supplemented with Gliricidia (●) (SEM ± 0.0015).

Plasma concentration of glucose reflecting the fact that plasma glucose normally is under very strict homeostatic control. It was observed that GER rate was positively related to N retention. As GER increased in animals in the MG group through GS to the MGGS group, N retention accordingly was increased (Fig. 3). The R^2 value was only 0.6572 indicated that the relationship was not strongly linear. It indicated that there is other factor influence the GER, such as fiber or carbohydrat content in the diet. Similar results[26] noticed that there was an increasing in concentration of blood glucose due to more glucogenic amino acids available for gluconeogenesis, when the protein from leguminouse leaves was added in the diet. An increasing in glucose concentration occured due to more by-pass protein and increased availability of glucogenic amino acids for glucose synthesis[27]. When sheep fed *Gliricidia sepium* as protein sources feed was increased[28]. It is clear that the amino acid contained in leguminous leaves has a role in improving glucose concentration in the plasma by providing glucose precursor for gluconeogensis. It is indicated that leguminous trees as good protein sources should have better amino acid and micro nutrient profiles with safe levels of anti-nutritional factors[29].

The low amount of DOMI by the MG sheep was reflected in the group's plasma glucose concentration profile (Fig. 1). The data presented in Figs. 1 and 2, suggest that glucose concentration in plasma would peak in 1.5 - 2.0 h immediately after feeding in sheep.

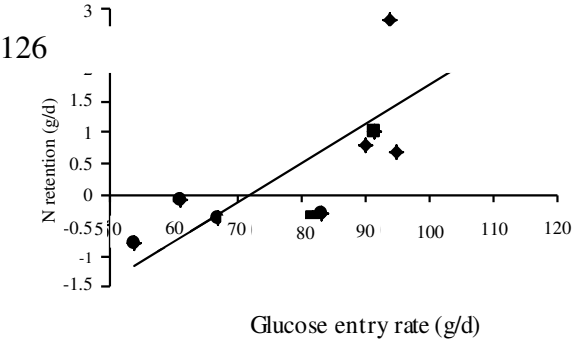

Fig. 3. Relationship between N retention and glucose entry rate in sheep fed Mitchell grass hay (●), Gliricidia (■) and hay supplemented with Gliricidia (♦). The relationship may be expressed as: $Y = 0.0634 X - 4.5852$ ($R^2 = 0.6572$), where Y is the N retention and X is the glucose entry rate.

Much of the increase in glucose concentration in plasma would be from propionate absorbed across the rumen wall into the bloodstream and used as a gluconeogenic substrate by the liver. Any contribution to glucose synthesis by gluconeogenic amino acids probably would occur after the initial 1.5 - 2.0 hours after feeding. The plateau in plasma glucose concentration, 1.5 - 2.0 hours after feeding (Fig. 1) and the maintenance of the plateau concentration for at least four hours after feeding, is consistent with the two-hourly feeding regimen adopted in the current experiment to establish equilibrium in animals under observation.

CONCLUSION

It is concluded from the results of the current experiment that the utilisation of GS by the ruminant animal may be improved by feeding it with a low quality feed at a ratio of 40:60 (GS:Low quality feed) to achieve an NI:DOMI ratio of 0.03 - 0.04. The improvement would be manifested in increased DOMI, with subsequent increase in GER or net protein deposition as might be expressed in positive N retention.

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