Glucose metabolism in cattle given sugar cane based diets supplemented with varying quantities of rice polishings

BY H. M. FERREIRO*, A. PRIEGO*, J. LOPEZ*, T. R. PRESTON* AND R. A. LENG†

Centro de Investigacion y Experimentacion Ganadera Chetumal, QR Mexico

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1. Glucose entry rates were measured with [2-^{3}H]glucose in groups of cattle given sugar-cane diets and between 0 and 1200 g rice polishings.

2. In the first experiment measurements of glucose metabolism were estimated in four animals (one of each being given 0, 400, 600 and 1000 g supplement) over 24 h using a repeated single injection at 6 h intervals and sampling blood for 3 h.

3. The results indicated that in a short time period of each isotope experiment relatively steady-state conditions existed since the plot of log specific radioactivity *v.* time was linear with a high correlation coefficient.

4. The pattern of glucose entry rates was variable over the 24 h period being highest shortly after feeding and then declining to quite low levels immediately before the next feed, 24 h later. However, the more rice polishings that were made available to the cattle, the higher the glucose entry rate at 4–7 h, and it remained higher for a longer time.

5. In the second experiment with nineteen animals there was a linear relationship between the glucose entry rate (measured 4–7 h after feeding) and the amount of rice polishings consumed by the animal.

6. The results suggest that glucose is being made available in quite large quantities from the supplement. Using the means of these estimates over 24 h to predict glucose entry rate on a daily basis, it is suggested that at least 50 % of the starch in the rice polishings was made available to the animal as glucose.

7. The results are discussed in relation to the suggestion that the availability of glucose may be a limiting nutrient in cattle given low-protein diets.

The utilization of sugar cane as the major food ingredient for cattle is becoming well established particularly in the Caribbean area (Preston, 1977) but has also been used in Australia, Thailand, Mauritius and parts of Africa (see Preston & Leng, 1978).

Utilization is limited by the requirements for expensive concentrate supplements; without these little growth is obtained. However, there is a linear increase in growth with supplements of rice polishings, growth being increased by 100 g/d for each 100 g rice polishings fed (Preston *et al.* 1976).

Examination of rumen function in cattle given sugar-cane diets with and without rice polishings indicated that the supplement had little or no effect on rumen fermentation and suggested that its effect may be mediated by providing nutrients postruminally (Valdez *et al.* 1977). Leng & Preston (1976) using stoichiometric principles suggested that a limitation to growth might be imposed by the availability of glucogenic precursors in cattle on these diets since rapidly-growing ruminants have a high glucose entry rate (T. J. Kempton & R. A. Leng, unpublished results; G. H. Smith, T. J. Kempton & R. A. Leng, unpublished results). It was postulated that considerable amounts of starch may have escaped fermentation in the rumen and contributed glucose directly to the animal. In order to study this hypothesis, estimates have been made of total glucose entry rates in cattle on sugar-cane-based diets given varying quantities of rice polishings.

* Present address: *Departamento de Investigacion y Estudios Superiores, Escuela de Medicina, Veterinaria y Zootecnia, Universidad del Yucatan, Mexico.*

† Present address: *Department of Biochemistry and Nutrition, University of New England, Armidale, NSW* 2351, *Australia.*

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MATERIALS AND METHODS

Experimental animals and diets

Experimental animals were Zebu and Zebu × Brown Swiss bulls weighing approximately 200 kg. These were held in groups of four animals per pen. For two weeks before the isotope experiments, the animals were tethered individually and were handled to accustom them to the experimental procedures. The animals had been on the diet for 3 months and were given freshly-harvested chopped whole sugar cane at 10.00 hours each day. Supplements of urea (to provide 40 g/kg sugar-cane dry matter (DM)), minerals and vitamins were mixed with the basal diet described previously by Preston *et al.* (1976). The animals were consuming on average 4·6 kg sugar-cane DM and there was no difference in intake of the basal diet between groups. Before the provision of sugar cane the animals were given a supplement of rice polishings which they consumed within 20 min of presentation. The animals had free access to water.

Experimental procedures

The day before an experiment the animals had a cannula placed in one jugular vein. On the day of the experiment, the animals were given their ration of rice polishings at approximately 10.00 hours, at 10.30 hours sugar cane was given in excess of their voluntary intake on the previous day.

In the first experiment the glucose metabolism was followed in one animal from each group over a 24 h period by injecting [2-^{3}H]glucose at intervals of 6 h and monitoring the specific radioactivity (SR) of glucose for 3 h. A total of four injections was administered to each animal in the 24 h period.

In the second experiment injections of isotope were made at 4 h after feeding. Animals from all groups were used and the total of experiments successfully completed was nineteen.

Isotope injections [2-^{3}H]glucose (200 μCi, 2 mg; Radiochemical Centre, Amersham, UK) contained in approximately 5 ml saline (9 g sodium chloride/l) was injected via the jugular vein cannula over approximately 1 min. The solution was washed in with a small amount of saline and blood was taken repeatedly into the syringe and re-injected. Eight blood samples were taken at 30 min intervals for up to 4 h post injection. Samples were immediately placed in cooled 15 ml centrifuge-tubes containing one drop of heparin (3000 units/ml). They were cooled in ice, centrifuged and the plasma separated and stored at -15 ° until analysed.

Chemical methods

Glucose was estimated by the glucose oxidase method of Hugget & Nixon (1957). Radioactivity in plasma glucose was estimated by scintillation spectrometry after isolating the glucose as the pentacetate derivative (Jones, 1965). Duplicate standards and blanks were included with every ten test samples to determine the background and efficiency of counting respectively. In one series, test samples (sixteen) were 'spiked' with a standard and recounted in order to check quenching and it was found that this was not significantly variable.

Glucose pentacetates were prepared from the injection solution in a similar way to that for preparation of samples and the injected amount calculated from the recovery of radioactivity in the pentacetate derivative.

Calculations

The SR of plasma glucose declined as a single exponential function with time (t) (see Fig. 1) of the form

$$SR_t = SR_0 e^{-mt}, \text{ where } m \text{ is the rate constant.}$$

Initially two methods were used to calculate the glucose entry rate over the experimental period. The first method used the area under the curve of SR with time (A_s):

$$\text{GER} = \frac{\text{I}}{A_s}$$

where GER is the glucose entry rate, A_s is the area under the curve calculated according to Katz *et al.* (1974) and I is the injected dose.

The second method assumed steady-state conditions and therefore:

$$\text{GER} = P_0 m, \text{ where } P_0 \text{ (Pool size)} = \frac{\text{I}}{\text{SR}_0}$$

A paired t test indicated no differences in the estimates made by the two methods and therefore in practice a linear relationship was fitted to the results, log SR *v.* time (from 0·5 h to 4 h) by the method of least squares. Correlation coefficients for the individual animals were between 0·9 and 0·99.

Glucose entry rate, pool size, half time ($t_{\frac{1}{2}}$) and space were calculated by standard procedures (see Judson & Leng, 1972).

RESULTS

SR of plasma glucose

The SR of plasma glucose declined as a single exponential function and the results for one animal injected with labelled glucose at intervals of 6 h through a 24 h period are shown in Fig. 1. Plasma glucose concentrations were fairly constant over the sampling periods after injection.

Measurements of glucose metabolism over 24 h in amimals on four levels of supplementation with rice polishings

One animal from each of the supplement groups given 0, 400, 600 or 1000 g of rice polishings was studied over 24 h. The results are shown in Table 1. There was a marked increase in total glucose entry rate as the intake of rice polishings increased, particularly at 4 – 7 h after ingestion of the supplement. The greater the quantity of rice polishings ingested the longer the glucose entry rates remained elevated compared to the animal on the basal diet. Immediately before feeding on the next day, glucose entry rates in all animals were low.

A feature of the results was the very variable glucose pool sizes and spaces of distribution of glucose in the animals over the 24 h period. On the basal diet at 10 and 16 h after feeding there appeared to be an increased glucose space whereas in all animals receiving supplement the glucose space in the 10 – 13 h period after feeding was almost half that at 4 – 7 h after feeding, the glucose space remained low relative to the initial measurements in the subsequent estimates (Table 1).

Glucose entry rates in cattle given varying quantities of rice polishings

Mean estimates of glucose pool size, half time, glucose entry rate and space are shown in Table 2 for animals given varying quantities of rice polishings between 0 and 1200 g/d. The mean pool sizes of glucose in cattle on the various levels of supplementation varied between 166 and 252 mg/kg body-weight but there was no apparent trend with supplements of rice polishings.

The half-time of glucose was not apparently related to the intake of rice polishings. There was, however, a close relationship between the glucose entry rate (Y(mg/min), Y' (mg/kg$^{0.75}$ per min)) and the intake of rice polishings (X) (g/d).

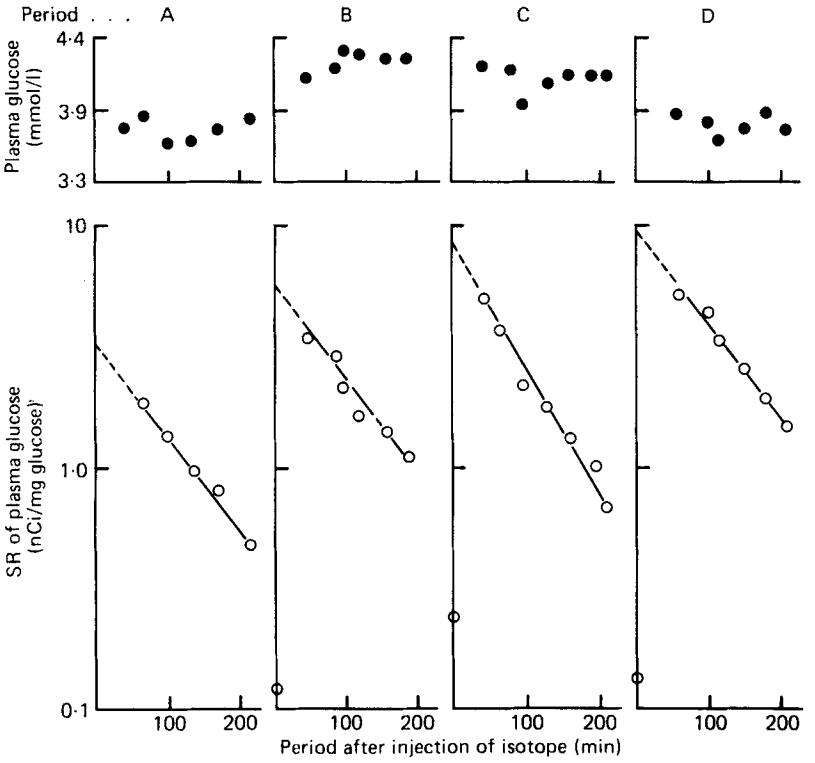

Fig. 1. Concentration (mmol/l) and specific radioactivity (SR; nCi/mg glucose) of plasma glucose in a bull (230 kg) after an injection of 200 μC: [2-^{3}H]glucose at 4 h (period A), 10 h (period B), 16 h (period C) and 22 h (period D) after feeding of the supplement. The basal diet was sugar cane supplemented with 600 g rice polishings. The SR of plasma glucose before the injection is shown at 10, 16 and 22 h. The SR was negligible relative to that after injection of isotope and was not taken into consideration in calculating the measurements of glucose metabolism.

Table 1. *Glucose metabolism at four periods/d in cattle given sugar-cane-based diets supplemented with* 0, 400, 600 *and* 1000 *g rice polishings*

([2-^{3}H]glucose was injected in one animal from each group 4, 10, 16, 22 h post feeding of the supplement. The animals had sugar cane available at all times)

Supplement (g/d)	Live wt (kg)	Period after feeding (h)	Plasma glucose (mmol/l)	Pool size		Half time (min)	Glucose entry rate		Space	
				g	mg/kg		mg/min	mg/kg$^{0.75}$ per min	l	g/kg live wt
0	211	4	4·4	27	128	64	294	5·3	34	160
		10	4·8	53	251	118	310	5·6	61	290
		16	4·9	50	237	115	148	2·7	56	270
		22	3·8	23	109	86	186	3·4	33	160
400	272	4	5·1	54	200	86	440	6·6	59	220
		10	4·7	35	129	68	362	5·4	42	150
		16	4·8	36	132	119	212	3·2	41	150
		22	4·2	17	63	71	164	2·5	23	80
600	230	4	3·8	59	256	77	530	9·0	86	370
		10	4·3	29	126	68	297	5·0	38	160
		16	4·2	24	104	60	274	4·6	32	140
		22	3·8	21	91	78	183	3·1	31	130
1000	241	4	4·8	72	299	73	539	8·8	84	350
		10	5·2	38	158	84	388	6·3	41	170
		16	4·8	38	158	66	394	6·4	44	180
		22	4·4	27	112	81	229	3·7	34	140

Table 2. *Glucose metabolism in cattle given sugar-cane-based diets supplemented with varying levels of rice polishings*

(Mean values with their standard errors are given for each group of animals. All measurements were made over the period 4–7 h after feeding the supplement. The cattle had sugar cane available at all times)

					Pool size						Glucose entry rate				Space			
			Plasma glucose (mmol/l)		g		mg/kg		Half time (min)		mg/min		$mg/kg^{0.75}$ per min		l		g/kg live wt	
No. of animals	Supplement (g/d)	Live wt (kg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3	0	213	4·3	0·52	44	3·1	208	17	98	15	360	18	6·4	0·5	59	5·5	280	13
2	200	222	4·4	0·04	42	1·7	190	23	79	1·5	370	21	6·5	0·8	53	2·7	240	32
3	400	215	4·2	0·29	36	0·83	166	4	67	5·8	378	44	6·7	0·8	47	2·7	220	13
3	600	220	4·2	0·09	44	9·2	204	50	73	12·1	423	47	7·5	1·1	59	12·9	270	71
1	800	220	3·7		55		252		95		404		7		83		380	
4	1000	237	4·3	0·34	47	6·4	198	28	69	10·6	479	11	7·9	0·2	62	12·0	260	51
3	1200	268	4·5	0·42	46	5·7	171	19	59	9·8	550	34	8·5	0·7	56	2·3	210	5

$$Y = 338 + 0{\cdot}161(\pm 0{\cdot}027)x \quad r^2\ 0{\cdot}62 \quad \text{residual SD (RSD) } 54$$

$$Y' = 6{\cdot}1 + 0{\cdot}002\ (\pm 0{\cdot}0005)x \quad r^2\ 0{\cdot}41 \quad \text{RSD } 1{\cdot}04$$

DISCUSSION

The glucose pool, total entry rate and space of distribution were highest 4 h after feeding the supplement and then declined over the 24 h period reaching very low levels immediately before the next feed. This pattern was not so clear in the animals on the basal diet (see Table 1, Fig. 1). The main effect of rice polishings was a large stimulation of glucose entry rate over the period 4–18 h after feeding the supplement. The stimulation was more apparent at the higher levels of intake of the rice polishings. The results indicate that considerable amounts of glucose were made available from the supplement since in addition to the results presented here rice polishings did not apparently increase fermentation rate in the rumen of cattle on this diet. (Valdez *et al.* 1977).

Taking the mean glucose entry rate over the 24 h period as representing the mean value of the four measurements made and subtracting the mean value for the animals on the basal diet then 230, 137 and 93 g glucose were apparently absorbed on the supplement levels of 1000, 600 and 400 g/d respectively. These amounts of glucose were approximately 230 g/kg supplement given, which is 600 g/kg total starch present in the supplement.

Examination of the glucose entry rates in the cattle at a time period (4–7 h) which can be assumed to represent peak rates of absorption, indicated that there was a significant relationship between these values and the amount of supplement given over a wide range of supplementation.

In these studies there were no differences in intake of sugar cane between the different levels of supplementation. This is in contrast to the effects on younger animals where the intake of sugar cane is increased by giving rice polishings (Preston *et al.* 1976). This eliminates the possibility that the glucose entry rate simply reflected an increase of intake of the basal diet. The supplement itself, however, raised total DM intake.

In contrast with the results reported on molasses-based diets (see Preston, 1972), protein meals of animal origin (fish meal and meat meal) have not stimulated food intake and growth on sugar-cane diets (see Preston, 1977), whereas rice polishings have consistently increased both these in young growing animals. This suggests that the first limiting nutrient on sugar-cane-based diets is not necessarily dietary protein that becomes available from the intestines but rather nutrients which give rise to an increased availability of glucose (energy?) from the small intestine.

One of the most noteworthy features of the experimental results was the extreme variation in glucose entry rates over the 24 h period. On the highest level of supplementation the peak value at 4–7 h after feeding was almost three times the minimum value recorded immediately before feeding on the following morning. The other interesting finding was the tendency for glucose to be distributed through a larger space when glucose entry rates were at their maximum.

It is not proven here that the extra glucose entering plasma in cattle on the basal diet plus supplement arises from the diet and not by increased gluconeogenesis in the liver. However, the findings strongly suggest that the effect on cattle of giving rice polishings on a sugar-cane-based diet is to increase the availability of glucose from nutrients digested in the small intestine and therefore by inference that the availability of glucose is a limiting factor to animal growth on this diet. However, nutrients that are directly available to the ruminant have a high digestible energy content (i.e. they are very efficiently used) and the effects of other energy nutrients that escape chemical alteration in the rumen must be examined before these

conclusions can be firmly established. This is important since the utilization of the protein and lipid of rice polishings is not known. The passage of large quantities of starch to the duodenum has recently been confirmed in cattle given this diet and cannulated at the duodenum (Elliott *et al.* 1978).

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