Effects of Dietary Propionate and Heat Exposure on Glucose Disappearance Rate and Insulin Secretion in Sheep

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Abstract Sheep were fed lucerne hay alone or supplemented with a physiological dose of calcium propionate (10 mmol/kg BW) and exposed to a thermoneutral (20°C) or a hot environment (30°C) in order to observe the effects of dietary propionate and of heat exposure on glucose disappearance rate and insulin response following the intravenous injection of glucose.

The blood glucose disappearance rate before feeding tended to be higher for lucerne hay with the calcium propionate diet than for the lucerne hay alone diet, but the blood glucose disappearance rate after feeding tended to be lower for lucerne hay with the calcium propionate diet than for the lucerne hay alone diet. Insulin secretion in response to glucose injection before feeding tended to be greater during heat exposure than under the thermoneutral condition regardless of diet, and after feeding it tended to be smaller for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet, regardless of air temperature. These results suggest that both the rate of blood glucose utilization and insulin secretory response to exogenous glucose are influenced more by dietary conditions than by environmental heat exposure.

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Key words : insulin, propionic acid, glucose disappearance rate, heat exposure, sheep

Blood glucose metabolism is known to be influenced by nutritional, physiological and environmental conditions¹⁻³⁾. Blood glucose turnover rate measured by the isotope dilution method decreased during heat exposure in shorn sheep³⁾. The rumen concentration of propionic acid, one of the major glucogenic substrates, was reported to decrease markedly during heat exposure⁴⁾. Therefore, dietary supplementation of propionic acid may improve the decreased blood glucose turnover rate during heat exposure.

Insulin is one of the important hormones controlling carbohydrate metabolism in both ruminant and non-ruminant animals. The insulin secretory response to glucose injection is reported to be altered by cold exposure⁵⁾, fatness⁶⁾, stage of lactation⁷⁾ and feeding⁸⁾ in ruminants. However, little attention has been focused on the insulin

response to glucose injection during heat exposure. Our previous report showed that insulin response to feeding was accelerated in sheep fed lucerne hay with a physiological level of calcium propionate (10 mmol/kg body weight (BW)) both in thermoneutral and heat exposure conditions⁹.

The objective of this experiment was to measure glucose disappearance rates and insulin responses following glucose injection in sheep which were fed the high propionate diet and exposed to heat.

Materials and Methods

Animals

Six crossbred (Suffolk × Corriedale) shorn sheep were used, aged from 2 to 3 yrs, weighing 24 to 44 kg. They were surgically prepared with a skin loop enclosing the left carotid artery and a rumen cannula under anesthesia with pentobarbitone sodium (25 mg/kg BW) at least one month before the experiment started. Animals were kept in metabolic cages in a controlled environment chamber at an air temperature of 18–22°C for more than a week before the experiment. Other animal treatments were almost the same as described previously⁹. Each sheep received the following four treatments: (1) 20 L: lucerne hay cube alone diet (fed at a level of 20 g/kg BW/day) in a thermoneutral environment (20°C); (2) 20 P: lucerne hay cube (16 g/kg BW/day) plus calcium propionate (10 mmol/kg BW/day) diet in a thermoneutral environment; (3) 30 L: lucerne hay cube alone diet in a hot environment (30°C); (4) 30 P: lucerne hay cube plus calcium propionate diet in a hot environment. Each treatment lasted for 2 weeks and the sequence of treatments was incompletely randomized. The lucerne hay cubes contained 8800 kJ digestible energy and 109 g crude protein per kilogram. The animals were fed these diets almost isoenergetically at 12:00 for 2 hrs, and water was given at 12:00 for 6 hrs. Other aspects of animal management were the same as described previously⁹.

Experimental procedures

The experiments were carried out on days 11 and 14 of each treatment. Polyethylene catheters were inserted into the jugular vein at least 5 days in advance and into the skin loop of the carotid artery at least 2 hrs before the experiment commenced.

Glucose (0.625 mmol/kg BW, 2.5 M solution) was injected through the jugular catheter for 1 min at 2 hrs before or 2 hrs after feeding on different experimental days (day 11 or 14) for each treatment. Blood samples (5 ml) were taken from the carotid catheter at -10, -5, 0, 5, 10, 15, 30, 45, 60 and 90 min, and rumen samples (10 ml) were taken through the rumen cannula at 0, 45 and 90 min after glucose injection. Blood and rumen samples were centrifuged in the cold (4°C) at $10,000 \times g$ for 10 min and the supernatants of blood and rumen fluids were stored at -25°C until analysis.

Analysis

Concentrations of glucose and insulin in plasma and volatile fatty acids (VFA) in the rumen fluid were measured as described previously⁹. The concentration of calcium

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in the rumen was determined by using a Calcium test kit (Wako, Osaka)¹⁰⁾.

Calculations

The glucose disappearance rate was calculated from the incremental concentrations of plasma glucose at 15, 30 and 45 min after glucose injection⁸⁾. The insulin area, which was the incremental area under the curve above the initial level for insulin concentrations in response to glucose, was calculated over 90 min after glucose injection. The significance of differences was evaluated via the Student's paired *t*-test which was used to compare preinjection means with values obtained after glucose injection and also to test the effects of the timing of glucose injection. Analysis of variance and F-test were used for comparisons between the treatments.

Results and Discussion

Physiological responses

Physiological responses, feed intake and water intake have been reported previously⁹⁾. Feed intake was less ($P < 0.01$) for the lucerne hay with calcium propionate diet (0.50 and 0.47 kg/day for 20 P and 30 P) than for the lucerne hay alone diet (0.65 and 0.67 kg/day for 20 L and 30 L), although the diet amounts offered to the sheep were less for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet because these diets were offered isoenergetically in the present experiment. The lucerne hay with calcium propionate diet might not have been palatable for sheep.

Rumen fluid

Table 1. Effects of dietary calcium propionate and heat exposure (30°C) on concentrations of total VFA, acetic acid, propionic acid, butyric acid and calcium in the rumen

Time of injection	Treatment	Total VFA		Acetic acid		Propionic acid		Butyric acid		Calcium			
		(mmol/l)										(mg/100ml)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Before feeding	20L	42	5	30	4	7	1	3	0	3	1		
	20P	35	2	26	2	5	0	2	0	6	2		
	30L	46	3	34	2	7	1	3	0	5	1		
	30P	42	5	30	4	7	1	3	0	6	2		
After feeding	20L	130	12	94	8	26	3	8	1	34	5		
	20P	145	4	69	4	70	4	6	0	95	10		
	30L	100	9	73	7	20	1	6	1	28	2		
	30P	108	11	55	6	47	6	4	1	74	13		
Main effect	before and after feeding	*		*		*		*		*			
	environment diet	* NS		* *		* *		NS *		NS *			

Mean values are presented, averaged over the three sampling times (0, 45 and 90 min after glucose injection).

* : $P < 0.05$

NS : not significant

20 : a thermoneutral environment (20°C), 30 : a hot environment (30°C), L : lucerne hay alone diet, P : lucerne hay with calcium propionate diet

Concentrations of calcium, total VFA and individual VFAs in the rumen during the experiment are given in Table 1. All concentrations were similar between treatments before feeding, but were much higher ($P < 0.05$) after feeding than before feeding. Concentrations of propionic acid and calcium in the rumen after feeding were much higher ($P < 0.05$) for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet. Concentrations of acetic acid in the rumen were lower for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet. Concentrations of total VFA in the rumen did not differ between the diets. The concentrations of propionic acid in the rumen after feeding were within the physiological range¹¹ in the present experiment.

The concentrations of total VFA, acetic acid and propionic acid in the rumen after feeding were lower ($P < 0.05$) during heat exposure than under the thermoneutral condition: nevertheless, feed intake did not differ between the environments (0.65, 0.50, 0.67 and 0.47 kg/day for 20 L, 20 P, 30 L and 30 P, respectively). The concentrations of butyric acid and calcium in the rumen tended to be lower during heat exposure than under the thermoneutral condition. These results may correlate with the increase in water intake during heat exposure.

Plasma constituents

Baseline concentrations of plasma glucose were similar between treatments before feeding (50.6, 46.9, 48.2 and 50.9 mg/100 ml for 20 L, 20 P, 30 L and 30 P, respectively), but tended to be higher for the lucerne hay with calcium propionate diet (51.9 and 50.1 mg/100 ml for 20 P and 30 P) than for the lucerne hay alone diet (44.8 and 48.6 mg/100 ml for 20 L and 30 L) after feeding (Fig. 1). Concentrations of plasma glucose after the intravenous injection of 0.625 mmol/kg BW of glucose increased ($P < 0.01$) rapidly, reaching a peak (91.7–104.4 mg/100 ml) at 5 min after glucose injection and decreasing gradually thereafter in all of the experiments. The extent of hyperglycemia induced by glucose injection was similar between treatments. The plasma glucose concentration before feeding almost returned to the initial values by 90 min after glucose injection. The blood glucose disappearance rate before feeding tended to be higher for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet, regardless of environmental temperature (Table 2). However, the feed treatments and environmental conditions in the present experiment did not significantly affect the blood glucose disappearance rate. The elevated plasma glucose concentration after glucose injection decreased slower after feeding than before feeding. Plasma glucose concentrations after feeding still remained higher at 90 min after glucose injection than the initial levels, the differences from the initial values being 10.4, 9.3, 11.1 and 12.9 mg/100 ml for 20 L, 20 P, 30 L and 30 P, respectively. Furthermore, the elevated plasma glucose concentrations after glucose injection decreased slower for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet after feeding. The glucose disappearance rate also tended to be lower for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet after feeding. Insulin action to glucose utilization may be reduced when large

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Fig. 1. Effects of dietary calcium propionate and heat exposure (30°C) on plasma glucose and insulin in response to intravenous injection of glucose (0.625 mmol/kg BW) at 2 hrs before feeding (B) and 2 hrs after feeding (A) in sheep. The treatments were as follows: 20 L: lucerne hay alone diet in a thermoneutral environment (○) ; 20 P: lucerne hay with calcium propionate diet in a thermoneutral environment (●) ; 30 L: lucerne hay alone diet in a hot environment (△) ; 30 P: lucerne hay with calcium propionate diet in a hot environment (▲).

amounts of nutrients containing insulintropic substrates are absorbed from the digestive tracts, especially in the case of the lucerne hay with calcium propionate diet.

Concerning the glucose disappearance rate of the injected glucose, no significant differences were observed between the treatments or between the timing of glucose injections in the present experiment. A more sensitive parameter of glucose metabolism, the blood glucose turnover rate, was not measured simultaneously in the present study. The glucose disappearance rate for the lucerne hay alone diet did not differ between before and after feeding, but for the lucerne hay with calcium propionate diet it tended to be lower after feeding than before feeding. KATZ and

Table 2. Effects of dietary calcium propionate and heat exposure (30°C) on insulin area* and glucose disappearance rate following glucose injection in sheep

Timing of injection	Treatment	Glucose disappearance rate (%/min)		Insulin area (mU/ml · 90 min)	
		Mean	SE	Mean	SE
Before feeding	20L	2.4	0.4	1.1	0.3
	20P	3.0	0.4	1.2	0.3
	30L	1.8	0.4	1.5	0.4
	30P	3.0	0.8	1.6	0.5
After feeding	20L	2.1	0.5	1.9	0.3
	20P	1.7	0.4	0.9	0.3
	30L	2.4	0.7	2.0	0.5
	30P	1.8	0.1	1.6	0.3

* Insulin area is the incremental area above the initial level over 90 min after glucose injection.

20 : a thermoneutral environment (20°C),

30 : a hot environment (30°C),

L : lucerne hay alone diet,

P : lucerne hay with calcium propionate diet

BERGMAN¹²⁾ demonstrated that hepatic glucose production in sheep increased after feeding. SASAKI *et al.*⁸⁾ also reported that the glucose disappearance rate in sheep was much higher during a meal. As far as the present experiment is concerned, however, it is likely that the glucose disappearance rate of injected glucose is not a very sensitive index of blood glucose metabolism, especially when exogenous glucogenic substrates are supplied additively to the diet. Therefore, there was no exact indication of whether the decreased blood glucose turnover rate during heat exposure was improved by the propionate supplementation or not.

Baseline concentrations of plasma insulin tended to be higher after feeding (22.4, 28.6, 23.7 and 27.1 μ U/ml for 20 L, 20 P, 30 L and 30 P, respectively) than before feeding (14.9, 14.0, 15.6 and 21.3 μ U/ml for 20 L, 20 P, 30 L and 30 P, respectively), and also tended to be higher during heat exposure than under the thermoneutral condition (Fig. 1). Plasma insulin responded significantly ($P < 0.05$) to the intravenous injection of glucose (0.625 mmol/kg BW) for all treatments. The peak concentrations of plasma insulin before feeding tended to be higher during heat exposure (82.5 and 84.0 μ U/ml at 10 min for 30 L and 30 P) than under the thermoneutral condition (55.3 and 54.2 μ U/ml at 5 min for 20 L and 20 P), regardless of the diet. The insulin area before feeding also tended to be larger during heat exposure than under the thermoneutral condition (Table 2). It was observed that insulin concentration during the initial stage after feeding tended to be greater during heat exposure than under the thermoneutral condition in sheep fed a diet of lucerne hay alone⁹⁾. However, DENBOW *et al.*⁷⁾ reported that both the baseline insulin concentrations and the magnitude of the insulin response to glucose in cattle were higher in spring than in summer. They suggested that lower insulin during summer was due to the result of decreased VFA.

VFA levels in the rumen before feeding, which did not differ between the treatments in the present experiment, may explain a part of this inconsistency.

The peak insulin concentrations induced by glucose injection after feeding tended to be lower for the lucerne hay with calcium propionate diet (60.6 and 66.6 $\mu\text{U/ml}$ for 20 P and 30 P) than for the lucerne hay alone diet (77.9 and 83.7 $\mu\text{U/ml}$ for 20 L and 30 L). The insulin area after feeding also tended to be smaller for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet. It was observed that insulin levels, which had reached a peak, decreased gradually in sheep fed the lucerne hay with calcium propionate (10 mmol/kg BW/day) even though propionic acid in the rumen still remained at a high level. WEEKES¹³⁾ administered a primed infusion of a VFA mixture into the rumen of sheep for 6 hrs and found a similar result. He suggested that the trigger for insulin secretion might be a change in the level of propionate rather than the absolute level of propionate (personal communication). It is concluded that a greater supply of insulinotropic substrates in the diet decreases the insulin responsiveness to glucose regardless of air temperature.

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めん羊におけるグルコース消失速度およびインスリン分泌に対する 飼料中プロピオン酸および暑熱の影響

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グルコースの静脈注入後のグルコース消失速度およびインスリン分泌反応に対する飼料中プロピオン酸および暑熱の影響を明らかにするために, めん羊にアルファルファヘイキューブ単一飼料あるいはアルファルファヘイキューブに生理的レベルのプロピオン酸カルシウム (10 mmol/kg BW) を添加した飼料 (高プロピオン酸飼料) を給与し, 常温 (20℃) あるいは暑熱 (30℃) に暴露した.

血液グルコース消失速度は採食前にはアルファルファヘイキューブ単一飼料よりも高プロピオン酸飼料の方が

高い傾向を示したが, 採食後には低い傾向を示した. グルコース注入に対するインスリン分泌反応は採食前には飼料に関係なく常温時よりも暑熱時の方が大きい傾向を示し, 採食後には環境温度に関係なくアルファルファヘイキューブ単一飼料よりも高プロピオン酸飼料の方が小さい傾向を示した. これらの結果から血液グルコースの利用速度および外因性グルコースに対するインスリン分泌反応はともに暑熱暴露よりもむしろ飼料条件に影響されると考えられた.

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