Effects of Dietary Propionate and Heat Exposure on Insulin Response to Feeding in Sheep

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Abstract Sheep were fed lucerne hay alone or supplemented with calcium propionate (10 mmol/kg BW) and exposed to a thermoneutral (20°C) or a hot environment (30°C) in order to clarify effects of physiological level of propionic acid in the rumen and heat exposure on insulin secretion in response to feeding. Total volatile fatty acids (VFA) concentrations in the rumen increased after feeding, with a particularly sharp increase in propionic acid levels for the lucerne hay with calcium propionate diet. Plasma glucose concentrations after feeding were higher for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet. Concentrations of plasma insulin for the lucerne hay alone diet responded to feeding biphasically, with a more rapid and marked response for the lucerne hay with calcium propionate diet in both the environments. The concentrations of plasma insulin at the initial stage after feeding tended to be higher during heat exposure than under thermoneutral conditions for the lucerne hay alone diet. There was little difference in plasma glucose concentration between the environments. These results suggest that administration of a physiological dose of propionic acid into the rumen causes insulin secretion in sheep. It is likely that plasma insulin level tends to be higher during heat exposure than in the thermoneutral environment but the insulin response to feeding did not differ between the environments under normal feeding conditions.


Key words: insulin, propionic acid, feeding, heat exposure, sheep

In ruminant animals most energy is supplied as VFA derived from fermentation by the rumen microorganisms. The ruminant's glucose requirement must depend on gluconeogenesis in the liver and kidney because little glucose is absorbed from the digestive tract under normal conditions in the ruminant. Propionic acid is the only VFA which can be metabolised to glucose and glycogen, and the most important single source of glucose in fed ruminants1).

Blood glucose metabolism was demonstrated to decrease during heat exposure in
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It is well-known that insulin, which is secreted from the islets of Langerhans in the pancreas, is the most important hormone to control carbohydrate metabolism in both ruminant and nonruminant animals. Therefore, plasma insulin level may be influenced by heat exposure.

VFA have been shown to stimulate insulin secretion in ruminants. It is still however uncertain whether a physiological dose of propionic acid stimulates insulin secretion or not in ruminants.

The objective of this experiment is to clarify this question by means of supplementation of propionate to the diet, rather than using intraruminal or intravascular infusions of propionate, as have been employed previously. Moreover, the insulin response to feeding during heat exposure was measured, because it may change in relation to the decreased blood glucose metabolism.

Materials and Methods

Animals

Six crossbred (Suffolk × Corriedale) shorn sheep, two ewes and four wethers, aged 2 to 3 yrs, and weighing 24 to 44 kg were used. They were surgically prepared with a skin loop enclosing the left carotid artery and a rumen cannula at least a 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 to accustom to the experimental procedures and surroundings and they were fed daily at 12:00. Water was given at 12:00 for 6 hrs.

Dietary treatments consisted of lucerne hay cubes alone (fed at a level of 20 g/kg body weight (BW)/day) or lucerne hay cubes (16 g/kg BW/day) plus calcium propionate (10 mmol/kg BW/day). Calcium propionate (Mitsubishi-Kasei Ltd. Tokyo) was added to the lucerne hay by mixing with 0.2 l of tap water. The amount of supplemented calcium propionate corresponded to the daily production of propionic acid in the rumen in sheep fed 900 g of dried grass and was about twice the amount of absorbed propionic acid in sheep fed 800 g of lucerne pellets daily. Sheep were fed these diets almost isoenergically, because plasma insulin levels in sheep are known to increase with the level of nutrient intake. The amount of diet offered was less for the lucerne hay with calcium propionate than for the lucerne hay alone. The lucerne hay cube contained 8800 kJ digestible energy and 109 g crude protein per kilogram. The room temperature in the chamber was maintained at 20 ± 2°C for a thermoneutral condition and elevated to 30 ± 2°C for a heat exposure condition. Therefore, the animals were exposed to either a thermoneutral (20 ± 2°C) or a hot environment (30 ± 2°C) for 2 weeks. Each sheep received the following four treatments: 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) and 30 P (lucerne hay with calcium propionate diet in a hot environment). Each treatment lasted for 2 weeks and the sequence of treatments was incompletely randomized. Therefore, the experiment lasted for 8 weeks.
Experimental procedure

Physiological responses were observed in the morning of the second week of each treatment. The experiment was carried out on day 8 of each treatment. A catheter was placed in the carotid artery of the skin loop at least 2 hrs before the experiment commenced. Blood samples (5 ml) were taken from the carotid catheter at -30, -15, 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min, and rumen fluid samples (10 ml) were taken through the rumen cannula at 0, 30, 60, 120, 180, 240 and 360 min after feeding. Immediately after centrifugation in the cold (4°C), the supernatant fluids of blood and rumen samples were stored at -25°C until analysis.

Analyses

The concentrations of VFA in the rumen fluid were measured by gas chromatography (Yanaco, Model G 80). Plasma glucose concentrations were measured enzymically\(^\text{17}\). Plasma insulin was assayed as described by SASAKI and TAKAHASHI\(^\text{18}\).

Statistics

The significance of difference was evaluated by Student’s paired \(t\)-test comparing prefeeding means with values obtained after feeding, and analysis of variance and \(F\)-test when comparing among the treatments.

Results and Discussion

Physiological responses

Increases in respiration rate \((P<0.01)\), water intake \((P<0.01)\) and rectal temperature \((P<0.01)\) were observed during heat exposure \((30L and 30P)\) (Table 1), as observed previously in heat exposed shorn sheep\(^2\). Feed intake was less \((P<0.01)\) when sheep recived the lucerne hay with calcium propionate diet \((20P and 30P)\) than for the lucerne hay alone diet \((20L and 30L)\), but intake was unchanged during heat exposure. The high propionate diet might not be palatable for sheep, although the weight of diet offered to the sheep was less for the lucerne hay with calcium propionate diet than for the lucerne hay alone diet in the present experiment. ANIL and FOBes\(^19\)

Table 1. Effects of heat exposure \((30°C)\) and dietary calcium propionate on physiological responses in sheep

<table>
<thead>
<tr>
<th>Treatment</th>
<th>20L</th>
<th>20P</th>
<th>30L</th>
<th>30P</th>
<th>SE</th>
<th>Temperature</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (kg/day)</td>
<td>0.65</td>
<td>0.50</td>
<td>0.67</td>
<td>0.47</td>
<td>0.03</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Water intake (/day)</td>
<td>3.1</td>
<td>3.3</td>
<td>5.8</td>
<td>4.8</td>
<td>0.4</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Respiration rate (min(^{-1}))</td>
<td>26</td>
<td>25</td>
<td>75</td>
<td>68</td>
<td>6</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Heart rate (min(^{-1}))</td>
<td>51</td>
<td>52</td>
<td>51</td>
<td>50</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>39,1</td>
<td>39,0</td>
<td>39,3</td>
<td>39,5</td>
<td>0,1</td>
<td>*</td>
<td></td>
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</table>

20L-lucerne hay alone diet in a thermoneutral environment; 20P-lucerne hay with calcium propionate diet in a thermoneutral environment; 30L-lucerne hay alone diet in a hot environment; 30P-lucerne hay with calcium propionate diet in a hot environment.

\(*P<0.01\)

Data are expressed as mean±standard error (SE) for means during the second week of each treatment.

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reported that an infusion of propionate into the portal vein (1.2 mmol/min) in sheep almost completely prevented feeding.

**Rumen fluid**

The concentration of total VFA in the rumen increased markedly after feeding, and reached peak values at 1 to 3 hrs after feeding (Table 2). These patterns of total VFA concentrations in the rumen obtained in the present experiment are in good agreement with the data for sheep fed 680 g of chopped lucerne\(^\text{20}\). The concentrations of total VFA in the rumen reached peak values faster when the lucerne hay with calcium propionate diet was fed than for the lucerne hay alone diet regardless of the air temperature. This is due to the marked increase in propionic acid concentration. The maximal concentrations of propionic acid in the rumen were 81 ± 8 and 64 ± 8 mmol/l (mean ± SE) at 20 P and 30 P respectively in the present experiment. Similar values were found in cows fed a high-grain, low-fibre diet\(^\text{21}\) and in sheep infused intraruminally with either a VFA mixture\(^\text{13}\) or with sodium propionate14). WEEKES and WEBSTER\(^\text{13}\) concluded that these VFA levels in the rumen remained within the physiological range. The concentration of total VFA at 2 hrs after feeding and thereafter was lower (P<0.05) during heat exposure (30 L and 30 P) than under thermoneutral conditions (20 L and 20 P). This was mainly due to the lower concentrations of propionic acid (P<0.05) and increased water intake (P<0.01) in the hot environment. The extent of the decreases in total VFA and propionic acid concentrations in the rumen during heat exposure in the present experiment, however, were smaller than those reported in nonlactating dairy cows exposed to 37.7° C\(^\text{22}\).

**Plasma constituents**

With the lucerne hay alone diet, concentrations of plasma glucose tended to decrease after feeding (Fig. 1). When the lucerne hay with calcium propionate diet was fed, however, the plasma glucose concentration increased after feeding and remained at higher levels than those for the lucerne hay alone diet regardless of the air temperature. The higher levels of plasma glucose may suggest that propionic acid derived from dietary calcium propionate is absorbed and hepatic gluconeogenesis from propionic acid is activated. JUDSON and LENG\(^\text{14}\) showed that glucose synthesized from propionic acid could account for 60–83% of the plasma glucose irreversible loss in sheep infused with propionate intraruminally at a rate of around 1 mmol/min for 5–6 hrs. Changes in the concentrations of plasma glucose after feeding during heat exposure were similar to those under thermoneutral conditions, as was the baseline plasma glucose concentration\(^\text{2}\).

For the lucerne hay alone diet, plasma insulin concentration increased (P < 0.05) transiently within 1 hr after feeding and increased again slightly thereafter in both the environments. These biphasic patterns of insulin secretion were not as clear as described by SASAKI et al.\(^\text{23}\). Plasma insulin concentrations during the first 3 hrs after feeding tended to be higher during heat exposure than in the thermoneutral environment, while the concentrations did not differ between the environments any longer at 4 hrs after feeding and thereafter (Fig. 1). The initial stage of feeding may
be adequate when comparing plasma insulin level during heat exposure with that under thermoneutral conditions, because a large amount of nutrients were absorbed after feeding and masked the environmental effect on plasma insulin. Release of both insulin and pancreatic glucagon in response to glycaemic stimuli was reported to depend upon the parasympathetic innervation to the gland in the calf. It was demonstrated that insulin secretion in response to a variety of stimuli was inhibited during cold exposure when the sympatho-adrenomedually system was activated.

From decreases in plasma FFA and T₄ concentrations described in a previous experiment and plasma insulin levels at the initial stage of feeding in the present experiment, we suggested that the parasympathetic system may have a greater activity than the sympatho-adrenomedally system during exposure to 30°C in shorn sheep.

The insulin response to feeding was much greater with the lucerne hay with calcium propionate diet than for the lucerne hay alone diet regardless of the air temperature. Plasma insulin levels for the lucerne hay with calcium propionate diet reached maximal values (20P 40.4 ± 8.1 μU/ml, 30P 36.8 ± 5.9 μU/ml) at 30-60 min after feeding and then decreased gradually. Significant differences were no longer found between the diets at 2 hrs after feeding and thereafter. It appears that the marked increase in plasma insulin levels after feeding the lucerne hay with calcium propionate diet is related to a physiological dose dietary calcium propionate. However, the route of the stimulation of insulin release still remains unknown from the present experiment. A number of studies concerning the insulin secretory response to VFA have been conducted with a variety of experimental procedures in ruminants, but the conclusions seem to be inconsistent. Stern et al. reported that insulin secretion failed to be stimulated by intraruminal infusion of a pharmacological dose of propionate in goats. Bassett found that a physiological dose of a VFA mixture given by continuous infusion into the rumen for 2 hrs in sheep failed to increase either insulin or glucagon concentrations, raising doubts about the physiological role of VFA as stimulators of insulin and glucagon secretions in ruminants. Trenkle concluded
that production of VFA in the rumen had some effects on the regulation of plasma insulin in ruminants. De Jong\textsuperscript{10} concluded that VFA, while not the sole controlling agents, might contribute to insulin secretion in the free-feeding goat. Weekes\textsuperscript{12} concluded that an intraruminal injection of VFA caused a transient release of insulin

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<table>
<thead>
<tr>
<th></th>
<th>Propionic acid</th>
<th>Butyric acid</th>
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<tr>
<td></td>
<td>20 L</td>
<td>20 P</td>
</tr>
<tr>
<td></td>
<td>(mmol/l)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>71</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>76</td>
<td>15</td>
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<tr>
<td>24</td>
<td>81</td>
<td>21</td>
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<tr>
<td>27</td>
<td>64</td>
<td>21</td>
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<td>26</td>
<td>55</td>
<td>18</td>
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<tr>
<td>23</td>
<td>42</td>
<td>16</td>
</tr>
</tbody>
</table>

**Fig. 1.** Effects of heat exposure (30°C) and dietary propionate on plasma glucose and insulin in response to feeding in sheep. 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 (▲). Mean values for 6 sheep.
in sheep and was as effective as a prolonged infusion of VFA.

The increased plasma insulin levels after feeding the lucerne hay with calcium propionate diet decreased gradually after the peak concentrations had been reached, while plasma glucose and rumen propionic acid remained at high levels. Perhaps, the responsiveness of insulin secretion to VFA, glucose or other insulinotropic substrates was reduced at that time. HIKOSAKA et al.\textsuperscript{27} reported that the insulin response to an intravenous infusion of glucose was higher during the first 2 hrs after feeding than during the last 2 hrs after feeding in sheep fed orchardgrass hay for 4 hrs once daily. Diurnal changes in plasma insulin relate to feeding under normal conditions\textsuperscript{8,28}. It is likely that the responsiveness of insulin release is reduced under conditions of \textit{ad libitum} or hourly feeding\textsuperscript{8,29}.

It is possible that calcium added to the diet might have activated insulin release in the present experiment, because glucose-induced insulin secretion was influenced by intracellular Ca\textsuperscript{2+} in mice \textit{in vitro}\textsuperscript{30}. The addition of 2 mM-EGTA to the perifusion medium was observed to inhibit both glucose- and butyrate-induced insulin release in sheep pancreas (Y. SASAKI, unpublished data). INOUE et al.\textsuperscript{31} showed that both intraduodenal and intravenous infusions of calcium stimulated the release of cholecystokinin, pancreatic polypeptide and gastrin in dogs. TAKAHASHI et al. (personal communication) observed that intravenous injections of gastrin and pancreozymin stimulated insulin secretion in sheep. Blood samples were therefore taken from two of the sheep when they were fed a diet of lucerne hay (20 g/kg BW/day) with added calcium carbonate (10 mmol/kg BW/day) under thermoneutral conditions (18–22°C). There was not such a marked increase in plasma insulin concentrations after feeding this diet as was observed when lucerne hay with calcium propionate was fed. It appears that the insulin response to the high calcium propionate diet is due to propionic acid in the present experiment.

In conclusion, it is suggested that a physiological dose of propionic acid in the rumen stimulates insulin secretion in sheep, but the route of insulin release still remains unknown. It is likely that plasma insulin level in sheep tends to be higher during heat exposure than in a thermoneutral environment but the insulin response to feeding did not differ between the environments under normal feeding conditions.

**Acknowledgements**

The authors are most grateful to Dr. T.E.C. WEEKES, University of Newcastle, for his kind advice on the manuscript.

**References**

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めん羊における採食に伴うインスリン分泌に対する
飼料中プロポシオン酸および暑熱の影響

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採食に伴うインスリン分泌に対する生理的レベルの第一胃内プロポシ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プロポシオン酸カルシウム（10 mmol/kg BW）を添加した飼料（高プロポシオン酸飼料）を給与し，常温（20℃）あるいは暑熱（30℃）に暴露した．採食に伴って第一胃内揮発性脂肪酸（VFA）濃度は増加し，特に高プロポシオン酸飼料給与時のプロポシオン酸濃度が著しく増加した．採食後の血漿グルコース濃度はアルファルファヘイキューブ単一飼料給与時よりも高プロポシオン酸飼料給与時の方が高にく推移した．血漿インスリン濃度は両環境ともアルファルファヘイキューブ単一飼料給与時に二相性の分泌を示し，高プロポシオン酸飼料給与時には速やかに著増した．アルファルファヘイキューブ単一飼料給与時において血漿インスリン濃度は採食後初期の段階では常温時よりも暑熱時に高い傾向を示した．血漿グルコース濃度は環境温度による差は認められなかった．以上の結果より，めん羊において生理的レベル内のプロポシオン酸の第一胃内投与はインスリン分泌を引き起こすと結論される．通常飼料給与時において血漿インスリン濃度は常温時よりも暑熱時に高い傾向にあるが採食に伴うインスリン分泌反応に差はなくかった．

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