EFFECT OF LEUCINE AND VALINE ON KETOGENESIS IN THE RUMINANT 1, 2

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SUMMARY
A study was made of the intermediary metabolism of L-leucine and D,L-valine in the goat. The ketogenic nature of leucine was demonstrated both in the fasted and fed animal. Following intraruminal or intravenous infusion, leucine disappeared very rapidly from the blood and actually depressed the plasma level of amino acids in the fasted animal.

Antiketogenic and glucogenic effects of valine were demonstrated following intraruminal or intravenous infusion.

Effects similar to those of leucine and valine were exhibited by their metabolic intermediates, the branched-chain volatile fatty acids. The marked ketogenic effect of isovaleric acid was demonstrated following intraruminal infusion. There was a triphasic response in blood glucose similar to that occurring when other ketogenic fatty acids are placed in the rumen.

Isobutyric acid exhibited marked glucogenic and antiketogenic properties when placed in the rumen.

Nutritionists have divided the amino acids into two groups, the glucogenic and ketogenic acids, based on their ability to produce glucose or ketone bodies within the animal body.

Numerous workers (10, 14) have observed the marked ketogenic effect of leucine with rat liver systems in vitro. Butts, Blunden, and Dunn (6) found that leucine contributed to acetone body formation when fed to the normal animal. A metabolic pathway proceeding through isovalerate as an intermediate has been proposed (2). This pathway supports the conclusion that, under the appropriate conditions (as in the diabetic organism), a mole of leucine could give rise to 1.5 moles of ketone bodies.

Valine has been observed to possess marked antiketogenic properties in animals suffering from ketosis (7, 12). Other workers have noted the gluconeogenic properties of valine in the normal and phlorizinized animal (16, 26). A pathway for the intermediary metabolism of valine with isobutyrate as an intermediate has been proposed (19).

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These metabolic effects have been observed in monogastric animals and little confirmatory research has been done in the ruminant. Annison and Pennington (1) observed that isovalerate, an intermediate in leucine metabolism, gave rise to acetate and also increased ketone body formation when incubated with sheep rumen epithelial tissue or liver slices. In similar studies with isobutyric acid, small amounts of propionic and acetic acids were produced and the antiketogenicity of this branched-chain volatile fatty acid was noted.

Since a metabolic ketosis is known to exist in the ruminant, it would seem desirable to find out what contribution, if any, the ketogenic amino acids make. This metabolic ketosis is characterized by a high blood level of ketone bodies and a low blood sugar. A metabolic disorder in humans has been found in recent years which is called leucine-induced hypoglycemia (8, 9). A very low blood sugar is observed following ingestion of meals high in leucine by sensitive subjects.

Thus, an experiment was devised to study the intermediary metabolism of a ketogenic amino acid (leucine) and a glucogenic amino acid (valine) in the ruminant.

EXPERIMENTAL PROCEDURE
The study consisted of two phases: (1) Effect of administration of the amino acids into rumen fistulae of fed goats; (2) intraruminal and intravenous administration of the amino acids or their metabolites to fasted goats. Table
1 summarizes the treatments imposed and the number of animals involved. The goats used were mature nonlactating females.

On the day of administration of the amino acid to the fed animals, nothing was fed when the goat was on hay alone, but the amino acid was administered immediately following the consumption of the allotment of pelleted corn on the other ration. Control values without amino acid administration were also determined for each ration (22). Blood samples were collected before feeding and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hr following the administration of the amino acid. These samples were collected from the jugular vein, using an oxalated syringe. Changes in various blood constituents were analyzed statistically by an analysis of variance for factorial experiments. This has been described earlier (22).

To understand more fully the absorption and metabolism of leucine and valine, both intraruminal and intravenous infusions were performed on goats fasted one to two days, so as to maximize the effect of each of the metabolites. For the intravenous portion of the study, each of the amino acids in solution was administered through an intravenous drip outfit into the jugular vein over a 10- to 15-min period. Ruminal punctures were made in another part of this study and solutions of each amino acid were dripped into the rumen from an intravenous drip outfit over a period of 30 min.

Each of the branched-chain volatile fatty acids was administered by syringe through a ruminal puncture.

Blood samples were taken immediately before and in the period following treatment in the studies with the fasted goats. The time and frequency of sampling varied with the method of application and the response expected from each metabolite.

Protein-free filtrates of the blood samples were prepared by the method of Folin and Wu (15). Blood ketones were distilled by the method of Behre and Benedict (3) and the acetone was determined by the method of Bloek and Bolling (5). The total ketone bodies are expressed as acetone. Blood sugars were determined by the method of Benedict (4).

To understand more fully the metabolism of the amino acids, blood urea and plasma a-amino N values were determined. Blood urea was determined on the whole blood by a microdiffusion method on the day of sampling (11). Urea standards were run each day to correct for variations in the urease activity.

Plasma was obtained for the determination of a-amino N content by centrifuging the blood for 10 min at 2,050 G's. Five milliliters of plasma and 10 ml of 10% TCA were used to prepare a protein-free filtrate. This was diluted tenfold and used in the analysis by a ninhydrin colorimetric method (27).

RESULTS AND DISCUSSION

Fed animals. L-leucine administration on both the hay and pelleted corn diets caused a significant increase in blood ketones (Table 2). The response on both diets following administration of leucine was quite similar (Figure 1). Since large amounts of isovaleric acid were present in the rumen at 4 hr (22), it is impossible to determine whether leucine or its metabolite, isovaleric acid, was responsible for this ketogenic effect. This ketogenic effect is similar to that in the nonruminant (24, 30).

Numerous workers (6, 12, 14) have found

| TABLE 1 |
| Details of methods of administration of the amino acids and the branched-chain volatile fatty acids |

<table>
<thead>
<tr>
<th>Diet</th>
<th>Metabolite</th>
<th>Amount (g)</th>
<th>Dilution with water (ml)</th>
<th>Method</th>
<th>No. of goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelleted corn</td>
<td>Control</td>
<td>L-Leucine 40</td>
<td>Fistula</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>40</td>
<td>Fistula</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>Control</td>
<td>L-Leucine 40</td>
<td>Fistula</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>40</td>
<td>Fistula</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pasted</td>
<td>L-Leucine</td>
<td>5</td>
<td>250 Intravenous infusion</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DL-Valine</td>
<td>5</td>
<td>80 Intravenous infusion</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>40</td>
<td>1,800 Intraruminal infusion</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DL-Valine</td>
<td>40</td>
<td>800 Intraruminal infusion</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isovaleric Acid</td>
<td>20</td>
<td>20 Ruminal puncture</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isobutyric Acid</td>
<td>20</td>
<td>20 Ruminal puncture</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2
Changes from 0 to 4 hr after treatment in blood glucose, ketone bodies, urea, and plasma α-amino N

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hay</th>
<th>Corn</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-0.3</td>
<td>10.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.4</td>
<td>7.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Valine</td>
<td>5.8</td>
<td>4.8</td>
<td>5.3</td>
</tr>
<tr>
<td>(mg %)</td>
<td>3.7</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Blood ketones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-0.3</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.7</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.3</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>(mg %)</td>
<td>Avg</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Blood urea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3</td>
<td>-6.5</td>
<td>-1.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.2</td>
<td>-2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Valine</td>
<td>0.8</td>
<td>-1.9</td>
<td>-0.6</td>
</tr>
<tr>
<td>(mg %)</td>
<td>Avg</td>
<td>-3.5</td>
<td></td>
</tr>
<tr>
<td>Plasma α-amino N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-1.0</td>
<td>-1.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.6</td>
<td>-1.6</td>
<td>-1.1</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.2</td>
<td>-0.9</td>
<td>-0.4</td>
</tr>
<tr>
<td>(mg %)</td>
<td>Avg</td>
<td>-1.2</td>
<td></td>
</tr>
</tbody>
</table>

* Differences between diets significant at (P < .05).
** Differences between diets significant at (P < .01).
'a'-'b' Data in vertical columns not followed by the same superscript are significantly different from each other (P < .05).

FIG. 1. Blood ketone levels following intraruminal administration of 40 g of leucine or valine to goats on hay or pelleted corn diets.

leucine to be ketogenic in perfusion and incubation studies with liver tissue, and also in studies in which leucine was fed to the intact animal. The work of Annison and Pennington (1) with rumen epithelial tissue suggests that it may be the intermediate isovaleric acid which is responsible.

Dl-valine administration had a tendency to depress blood ketone levels on the pelleted corn ration (Figure 1). This effect, however, was not statistically significant (Table 2). It is impossible to determine whether valine or its metabolite, isobutyric acid, was responsible for this antiketogenic effect.

Annison and Pennington (1) observed that isobutyric acid was antiketogenic in incubations with liver or rumen epithelial tissue obtained from the sheep. This confirms monogastric data obtained from animals with elevated ketone body levels (24). Similar work with phlorizinized and ketotic animals has shown the antiketogenic effect of valine (7, 12).

Pelleted corn increased the concentration of ketone bodies in the blood 6 hr after feeding on the control ration (Figure 1). This appears to be related to an increased level of ketogenic butyric acid in the rumen (22). The increase in butyric acid was quite marked compared to the increase in propionic acid and thus more than cancelled any antiketogenic effect of propionate. Jorgensen and Schultz (18) also observed that pelleted corn rations tended to increase blood ketone levels.

The feeding of pelleted corn significantly decreased blood urea concentrations at 4 hr on all treatments (Table 2). This effect is probably related to the low pH in the rumen of the animals fed this ration (22). Preston, Brener, and Pfander (23) observed that urea-N in the blood was decreased markedly by pelleted corn feeding. These workers suggested that the decrease in the blood urea after feeding may indicate a shortage of rumen soluble N and the passage of greater amounts of blood urea.
into the rumen, since a marked decrease in rumen NH₃-N was generally also observed following feeding. The presence of soluble carbohydrate stimulates ammonia utilization by the rumen microorganisms. This increased utilization of soluble N for microbial protein synthesis, rather than a decreased production of ammonia on the pelleted corn diets, probably accounts for the decreased blood urea. The interruption of the urea cycle on the pelleted corn ration is apparent.

The feeding of pelleted corn significantly lowered the plasma α-amino N level when compared to animals not fed on the hay diet. This effect is probably due to stimulation of protein synthesis.

Administration of valine tended to alleviate the depression of plasma α-amino N on both rations when compared to either leucine administration or the control (P < .07). Possible reasons for this effect will be discussed when reference is made to the intraruminal infusion of valine.

Infusion studies on fasted animals. The effect of the intravenous administration of each amino acid on changes in various blood constituents appears in Figure 2. Dl-valine raised the blood glucose level appreciably in the 4 hr following administration. This resembles the data obtained with monogastric animals showing the glueogenic nature of valine (16, 26).

The blood glucose level showed a small but negligible increase following intravenous leucine administration at a dose of 0.12 g/kg of body weight. This confirms data obtained with human subjects, that leucine has no depressing effect on the blood glucose of the normal individual (8). McArthur, Kirtley, and Waife (21) observed that normal human subjects and dogs responded to L-leucine with hypoglycemia when it was given orally in a large enough dose (approximately 750 mg/kg). These workers found that rats failed to respond at this dose level. At the dosage level used in this study, L-leucine did not produce hypoglycemia in the goat.

A small rise in blood ketone bodies immediately followed intravenous leucine infusion (Figure 2). The ketogenic nature of this amino acid has been known for a long time (6, 10, 14). The lack of a marked ketogenic effect may be explained by the rather small and temporary increase in plasma α-amino N. The plasma α-amino N level had returned to normal by 2 hr after leucine administration. After administration of valine, the α-amino N level at 4 hr was still above that of the zero hour. Other workers have observed very slight or no increases in blood amino nitrogen following the administration of leucine orally. Rogers, Spolter, and Harper (25) noted that oral leucine caused a drop in plasma isoleucine and valine concentrations, which suggests an antagonism between the other branched-chain amino acids and leucine. Hier (17) observed that, when leucine was given orally, plasma levels of arginine, isoleucine, methionine, phenylalanine, threonine, tryrosine, and valine were decreased. The rapid disappearance of leucine as indicated by the plasma α-amino N content and the relatively small increase in blood ketones suggest the participation of leucine itself in some synthetic process such as protein synthesis when given intravenously.

The effect of 0.5 hr intraruminal infusion of leucine and valine on changes in various blood constituents is given in Figure 3. Immediately following the infusion of valine, a marked increase in plasma α-amino N content occurred. Similar infusion of leucine had a depressing effect on α-amino N level in the plasma. These effects are quite similar to those just described for the intravenous infusion of each of the amino acids. This would indicate that some absorption of the amino acids is taking place within the rumen. Lewis and Emery (20) administered L-lysine through the fistula of a cow and observed that the plasma level of lysine
was markedly increased 4 hr after administration. Demaux et al. (13), by isolating a portion of the digestive tract of sheep in vivo, showed an increase in $\alpha$-amino N content in the blood perfusing the rumen. These experiments indicate that the rumen wall is permeable to amino acids and is a site for their absorption. This may account for the effect of valine in raising the depressed $\alpha$-amino N level of the plasma on both the hay and pelleted corn diets when compared to the control or leucine ($P < .07$).

The ketogenicity of leucine and glucogenicity of valine were again demonstrated (Figure 3). The administration of valine tended to reduce blood ketones in the period following the infusion. This confirms findings obtained with nonruminant animals (7, 12). These effects are probably due in part to the branched-chain fatty acids which would be produced by deamination and decarboxylation of the amino acids within the rumen (22). This effect should be less in these animals fasted for two days than in the fed animal.

The changes in blood glucose and ketone levels following the administration of the branched-chain fatty acids into the rumen are given in Figure 4. Following isovaleric acid administration, large increases in blood ketone bodies were noted. Numerous workers have observed similar effects with this branched-chain acid in the nonruminant (24, 30). Annison and Pennington (1) observed that with incubations of sheep rumen epithelial tissue and liver slices, isovalerate increased ketone body formation. Isovaleric acid is accepted as an intermediate in leucine metabolism and can account for its ketogenic properties.

The triphasic blood glucose response following isovaleric acid administration (Figure 4) is quite similar to that found when butyric and caproic acids are intraruminally administered (28). This is characterized by an initial increase in blood glucose, then a decrease, and finally an increase to higher-than-normal levels again at about 3 hr (28). All of these acids are nonglucose-forming in nature, so it is difficult to explain this phenomenon.

Administration of isobutyric acid intraruminally was followed by large increases in blood glucose, which confirms data obtained with the nonruminant (24). Annison and Pennington (1), in their incubations with sheep rumen epithelial tissue and liver slices, observed that isobutyrate gave rise to propionate. The observed antiketogenic effect of isobutyric acid (Figure 4) may be due to its metabolite, propionic acid, rather than a direct effect of isobutyric acid. Propionate has marked antiketogenic properties in the ruminant (29).
REFERENCES


