References


Caproic Acid and Ethanol in Ruminal Ingesta of Cattle Receiving Purified Diets

It has long been established that the major sources of energy for ruminants are volatile fatty acids (VFA), mainly acetic, propionic, and butyric acids occurring as end products of the rumen fermentation of the food carbohydrate. In addition, small quantities of valeric acid normally occur together with isocids of butyric and valeric acids.

During gas-liquid chromatographic partition (flame ionization) of rumen contents from dairy cows given a purified diet containing isolated soy protein or urea as sources of nitrogen (for composition of diets see Table 1), large quantities of an additional longer-chain acid identified as n-caproic acid were observed. The quantities observed, together with the other VFA's and rumen pH, are given in Table 2. It can be seen that these diets also gave rise to large proportions of n-valeric acid.

The experimental animals were two pairs of identical twins 45, 46 and 47, 48. The natural ration given to Cow 48 was, in percentage: cracked corn, 63.0, chopped alfalfa hay, 12.5, chopped timothy hay, 12.5, linseed meal, 5.0, cottonseed meal, 5.0, dicalcium-phosphate, 1.0, and trace-mineral salt, 1.0. During trials the cows were fed ad libitum and they consumed daily between 8 and 12 kg of feed. The unenated feed was recorded at 0700 hr and new feed given at 0700 an at 1600 hr. Further observa-

TABLE 1

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet S</th>
<th>Diet U</th>
<th>Diet SU</th>
</tr>
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<tr>
<td>Cornstarch</td>
<td>21.8</td>
<td>26.9</td>
<td>34.2</td>
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<td>Glucose monohydrate</td>
<td>21.8</td>
<td>26.9</td>
<td>21.6</td>
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<td>Wood pulp</td>
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<td>30.0</td>
<td>30.0</td>
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<tr>
<td>Urea</td>
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<td>4.7</td>
<td>4.2</td>
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<tr>
<td>Isolated soy protein</td>
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<td>14.9</td>
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<tr>
<td>Minerals</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Refined soybean oil</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Fed as 16-mm pellets.

b Finely ground.

c Mineral mixture contained CaHPO₄, 49.897%; K₂CO₃, 28.737%; MgSO₄, 11.010%; NaCl, 8.478%; FeSO₄, 0.750%; MnSO₄·H₂O, 0.112%; Na₂HPO₄·10 H₂O, 0.361%; ZnSO₄·7H₂O, 0.529%; CuCl₂, 0.030%; KI, 0.003%; CaCl₂·6H₂O, 0.001%; MoO₃, 0.001%; Na₂SeO₃, 0.001%. *Supplied per kilogram of diet: Vitamin A, 8,800 USP units; Vitamin D, 1,100 USP units; Vitamin E, 2 IU.

The type of fermentation of these rations was characterized by very low proportions of acetic acid and high proportions of propionic and butyric acids. However, the most interesting aspects are the large proportions of n-valeric and n-caproic acids. There were significant
TABLE 2
Average pH, total concentration, and molar proportion of volatile fatty acids in rumen contents of animals receiving experimental diets

Sampling time, 1100 h

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Diet</th>
<th>No. of samples</th>
<th>pH</th>
<th>Total volatile fatty acids (meq/liter)</th>
<th>Acetic Acid</th>
<th>Pro- pionic Acid</th>
<th>Butyric Acid</th>
<th>Valeric Acid</th>
<th>Caproic Acid</th>
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<td>45 S</td>
<td>2</td>
<td>6.0</td>
<td>167.9</td>
<td>35.1</td>
<td>28.6</td>
<td>29.5</td>
<td>13.7</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>46 S</td>
<td>2</td>
<td>6.4</td>
<td>166.2</td>
<td>43.3</td>
<td>29.5</td>
<td>16.8</td>
<td>8.8</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>47 S</td>
<td>8</td>
<td>6.2</td>
<td>130.1</td>
<td>46.3</td>
<td>28.0</td>
<td>31.7</td>
<td>6.5</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>48 Natural</td>
<td>10</td>
<td>6.5</td>
<td>101.5</td>
<td>58.2</td>
<td>26.0</td>
<td>13.0</td>
<td>2.8</td>
<td>Traces</td>
<td></td>
</tr>
</tbody>
</table>

* Including iso-butyric acid.
^ Including iso-valeric acid.

TABLE 3
pH, total concentration, and molar proportions of volatile fatty acids in the rumen contents in relation to time after feeding

Feeding times were 0600 and 1600 hr

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Diet</th>
<th>Time of day (hr)</th>
<th>pH</th>
<th>Total volatile fatty acids (meq/liter)</th>
<th>Acetic Acid</th>
<th>Propionic Acid</th>
<th>Butyric Acid</th>
<th>Valeric Acid</th>
<th>Caproic Acid</th>
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<td>2</td>
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<td>140.1</td>
<td>42.2</td>
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<td>1000</td>
<td>1600</td>
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<td>159.5</td>
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<td>24.2</td>
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<td>27.0</td>
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<tr>
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<td>75.3</td>
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<td>33.8</td>
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<td>7.0</td>
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</tbody>
</table>

* Including iso-butyric acid.
^ Including iso-valeric acid.

TABLE 4
Energy proportions of volatile fatty acids calculated from average molar proportions obtained at intervals after feeding

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Diet</th>
<th>Acetic Acid (%)</th>
<th>Propionic Acid (%)</th>
<th>Butyric Acid (%)</th>
<th>Valeric Acid (%)</th>
<th>Caproic Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 S</td>
<td>21.3</td>
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<td>29.2</td>
<td>8.1</td>
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<td></td>
</tr>
<tr>
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<td>30.1</td>
<td>18.4</td>
<td>15.0</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>47 SU</td>
<td>28.4</td>
<td>34.8</td>
<td>20.3</td>
<td>14.8</td>
<td>1.7</td>
<td></td>
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<tr>
<td>Keys/mole(4)</td>
<td>269</td>
<td>367</td>
<td>524</td>
<td>682</td>
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differences (P < 0.05) in the proportions of caproic acid with time after feeding. There were differences (P < 0.01) between cows in the proportions of acetic, propionic, butyric, and caproic acid (P < 0.01). This was particularly apparent with the proportions of caproic acid.

The importance of higher acids as energy sources here is better illustrated when expressed as their caloric proportions of the VFA mixture, a fact often overlooked. In Table 4, the average proportions of energy have been calculated from results given in Table 3, which indeed emphasizes the significance of the higher acids to the host animal.

The large proportions of valeric acid, and especially that of caproic acid, are surprising since, to the authors' knowledge, few reports of these quantities have occurred. However, on overfeeding with wheat Allison et al. (1) did find valeric and caproic acid concomitant to ethanol.

In studying the nutrition of the anaerobe *Clostridium kluyveri*, Bornstein and Barker (2) found that this organism, grown on ethanol in the presence of acetate, produced butyric and caproic acids, and that the quantities of butyric and caproic acids formed depended upon the ratio of ethanol to acetate in the substrate. They demonstrated that the reaction was an oxido-reduction reaction, during which ethanol was oxidized to acetic acid and acetic acid reduced to butyrate or caproate. They also showed that when the organism was grown in the presence of propionate and ethanol it produced large quantities of valeric acid.

The results here suggest the possibility of a similar reaction occurring in the rumen, with ethanol as an intermediate. It is of interest that the largest quantities of valeric acid occurred when the proportions of propionic acid were greatest (Cows 46 and 47), whereas the largest quantities of caproic acid coincided with large quantities of butyric acid (Cow 45).

To investigate the possible presence of alcohol in the rumen fluid, which may serve as an electron donor in the synthesis of butyric and caproic acids, additional rumen samples were obtained from animals receiving the same rations.

Samples of rumen fluid (100 ml) were made alkaline to pH 7.5 and distilled at 50 C. The distillate was boiled with K$_2$Cr$_2$O$_7$ (134 g/liter) and 10 N H$_2$SO$_4$ for 2 hr: the reaction mixtures were shown by gas-liquid chromatography to contain small quantities of acetic acid not present in the distillate prior to the above treatment. Following this observation, the rumen samples were analyzed for alcohols, using gas-liquid chromatography. The column was packed with 20% tetrahydroethylenediamine (THEED) 60-80-mesh chromosorb W (1). The carrier gas was N$_2$, column temp 100 C, and injection temperature 135 C. Using this procedure, the ethanol peak was very distinct and easily distinguished from isopropanol.

Results of this analysis are given in Table 5. There was a significant time-of-feeding effect in concentration of ethanol (P < 0.05), as observed for caproic acid, the highest concentrations noted shortly after feeding. Also, small quantities of propanol and butanol were detected in some samples. The sample obtained at 1800 hr from Cow 45 contained, for instance, 2.30 and 0.55 meq/liter of propanol and butanol, respectively. Moonaw and Hungate (6) reported that ethanol was metabolized very slowly when added to rumen content in vitro. In this experiment, ethanol disappeared rapidly with time after feeding, indicating either rapid metabolism or absorption.

Although the concentration of ethanol was highest shortly after feeding, as was the concentration of valeric and caproic acids, the association is not necessarily one of cause and effect. However, results of Bornstein and Barker (2) might point in this direction. The organisms involved here are not known. However, recently Slyter (7) isolated organisms from animals fed similar rations which produced large quantities of C$_3$, C$_4$, and C$_5$ acids. These organisms were presumptively identified as *Peptostreptococcus elsdenii*, also shown to produce longer-chain acids, with pyruvate and lactate as sources of acetyl-CoA and electrons (3). Lactic acid analysis was not made in this experiment, and the possibility of lactate being the source of electrons and acetyl Co-A rather than ethanol cannot be dismissed. In studying the effect of overfeeding sheep with wheat starch, Allison et al. (1) also noted lactic acid occurring simultaneously with ethanol.

It is possible that a more complete understanding of factors involved in condensation reactions between VFA might lead to methods of preserving a high milk fat content with cows given high-concentrate rations. Huber et al.

### TABLE 5

Concentrations of ethanol in rumen contents in relation to time after feeding

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Diet</th>
<th>0800</th>
<th>1000</th>
<th>1200</th>
<th>1400</th>
<th>1600</th>
<th>1800</th>
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<tr>
<td>45</td>
<td>S</td>
<td>3.2</td>
<td>3.9</td>
<td>2.7</td>
<td>2.8</td>
<td>1.6</td>
<td>4.7</td>
</tr>
<tr>
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<td>U</td>
<td>5.7</td>
<td>8.6</td>
<td>2.9</td>
<td>1.0</td>
<td>1.1</td>
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<tr>
<td>47</td>
<td>SU</td>
<td>3.9</td>
<td>0.4</td>
<td>0.8</td>
<td>0.3</td>
<td>1.4</td>
<td>2.9</td>
</tr>
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</table>

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(5) recently demonstrated that a high-concen-
trate ration which did not result in significant 
reduction in milk fat content was also a ration 
where there was a high proportion of butyric 
acid in the ruminal ingesta. Furthermore, gains 
in fermentation efficiency could result if excess 
hydrogen in the rumen could be directed to-
ward reducing acetic acid to ethanol and 
longer-chain acids, rather than being directed 
toward reducing carbon dioxide to methane.

E. R. ORSKOV, 1 W. P. FLATT, 
P. W. MOE, and R. R. OLTJEN
Animal Husbandry Research Division, 
USDA, Beltsville, Maryland

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from the Carlsberg Foundation, Copenhagen.

Incorporation of Radioactive Carbon from Glucose or Amino 
Acids by Rumen Microorganisms

Utilization of exogenous amino acids for 
protein synthesis by rumen microorganisms is 
an important question in the nitrogen metab-
olism of these organisms. The relatively slow 
hydrolysis of feedstuff protein, except for 
 casein, by rumen microorganisms (1) suggests 
that feedstuff proteins are not an efficient 
source of amino acids. Working with pure 
cultures of rumen bacteria, Bryant and Robin-
son (2) concluded that these organisms pre-
ferred to synthesize their cellular constituents 
from carbon sources other than amino acids. 
Similarly, the direct incorporation of labeled 
glutamic acid or aspartic acid into rumen mi-
crobial protein by the mixed rumen population 
was found by Portugal and Sutherland 
(3) to be relatively small. In fact, these au-
thors concluded that amino acids in the mi-
crobial protein must come almost exclusively 
from de novo synthesis.

The following experiment was designed to 
test the relative efficiency of incorporation of 
radioactive carbon from glucose or amino acids 
into trichloracetic acid-precipitable material 
by rumen microorganisms.

Experimental Procedure
Rumen samples were obtained from steers 

1 Published with the approval of the Director 
as Paper no. 1979, Journal Series, Nebraska Agri-
cultural Experiment Station. Project 15-10 of 
the Department of Biochemistry and Nutrition 
contributing to Regional Research Project NC-63.

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