ESTIMATION OF STOICHIOMETRIC PARAMETERS FOR RUMEN FERMENTATION OF ROUGHAGE AND CONCENTRATE DIETS

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Summary

A biochemical model coupled with an iterative nonlinear least-squares program was used to estimate stoichiometric parameters for fermentation of soluble carbohydrate, starch, hemicellulose, cellulose and protein in the rumen. Fermentation parameters were deduced for two sets of literature data, on (1) a roughage group of 60 diets fed to 137 animals and (2) a concentrate group of 48 diets fed to 374 animals. Soluble carbohydrate and starch fermentation parameters were much different for the roughage and concentrate groups. Differences were also noted in fermentation parameters for hemicellulose and cellulose. Model sensitivity to imposed changes of iteratively deduced parameters supported the conclusion that the estimated values are unique for all substrates except protein. In vitro estimates of isolated substrate fermentation from the literature agreed favorably with those indicated for hemicellulose. Cellulose fermentation was estimated to yield an acetate to propionate ratio of 7.8 to 13.1:1. This was higher than estimates in the literature of 1.3 to 3.6:1. Predicted fermentation patterns were within the range of normal biological variation noted for actual patterns. Systematic error components were identified as lignin plus ash as a percentage of feed dry matter and percentage roughage in the diet for the roughage and concentrate groups, respectively. Significant bias remained only in the roughage group for acetate and butyrate. Predicted vs observed acetate, propionate and butyrate production, for diets where comparison data were available, had slopes (correlation coefficients) of .84 (.98), 1.03 (.97) and .94 (.98), respectively. By this method, products of fermentation were estimated with nearly the same accuracy with which they can be measured experimentally. (Key Words: Rumen, Fermentation, Parameters, Stoichiometry, Model.)

Introduction

Direct experimental data on quantitative relationships among chemical components of ruminant feeds and products of fermentation are very limited. Both the inability to isolate chemical components in their native states and the overall complexity of ruminant digestion have stifled progress in this area (Baldwin et al., 1977).

Koong et al. (1975) described an alternative approach to the problem of relating fermentation products to diet composition. They proposed a model in which metabolic flux and stoichiometric parameters for alternate pathways in rumen fermentation were estimated by an iterative nonlinear least-squares method. In the sample problem they presented, model input consisted of the amounts of soluble carbohydrate, hemicellulose, cellulose and protein digested in the rumens of eight sheep fed white clover. Initial model outputs were bacterial cell growth and acetate, propionate, butyrate, valerate, methane and carbon dioxide production. This initial output was based on an arbitrary set of values for the unknown stoichiometric parameters. These parameters were then systematically adjusted to minimize the error term calculated when model outputs were compared to experimental data.

Objectives of this study were to accumulate the large data mass needed for iterative estimation of the stoichiometric parameter values and to examine the deduced parameters for uniqueness and variability.

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Materials and Methods

Data were gathered from the literature on molar productions of VFA with various diets. Considerable data of this nature are available for a few standard forages. These data alone, however, were not sufficient for our purposes. Protocols of the experiments varied tremendously. Complications were encountered when gross VFA production rates were corrected for rates of interconversion of isotopically labeled VFA used in the experiment. Most important, the range of diet composition studied was too narrow. The model of Koong et al. (1975), therefore, was altered to fit molar proportions of VFA rather than moles of each VFA produced. Because molar proportions of acetate, propionate, butyrate and valerate are commonly measured and reported, this change greatly expanded the available data mass. The VFA pattern is also known to be less sensitive than production rate to site and time of sampling.

Two groups of data were assembled from the literature. The first set consisted of information on 60 over 50% roughage diets. The data represented results on 137 animals from 20 studies (Stewart et al., 1958; Leng and Leonard, 1965; Gray et al., 1967; Hogan and Weston, 1967, 1969; Weston and Hogan, 1967, 1971; Corbett et al., 1969; Beever et al., 1971; Thomson et al., 1972; Chaturvedi et al., 1973 a, b; Punj et al., 1974; Egan et al., 1975; Harrison et al., 1975; Walker et al., 1975; Besecke and Mohme, 1976; Van Der Walt and Briel, 1976; Hume, 1977; Van Der Walt, 1977). The second group represented data on 48, mostly concentrate diets fed to 374 experimental animals and reported in 18 papers (Balch and Rowland, 1957; Woods and Luther, 1962; Oltjen and Davis, 1965; Oltjen et al., 1965; Thompson et al., 1965; Clanton and Woods, 1966; Weiss et al., 1967; Sutton, 1969; Sutton and Johnson, 1969; Franks et al., 1972; Utley and McCormick, 1972, 1975; De Vuyst et al., 1974; Stanley et al., 1975; White et al., 1975; Besecke and Mohme, 1976; Barry et al., 1977; Oshio et al., 1977).

Use of data from diets containing concentrates necessitated the inclusion of starch as a substrate in the model. Thus, model inputs were amounts of soluble carbohydrate, starch, hemicellulose, cellulose and protein digested in the rumen (mol/d).

Substrate composition of each diet as a percentage of dry matter was tabulated as reported in the original study or approximated from related work when detailed chemical analyses were not available. Feed intake data and the molecular weights of applicable substrates were then used to calculate amounts of substrate available for digestion (mol/d). Finally, amounts of each substrate digested in the rumen were estimated by application of rumen digestion coefficients from the original or related studies.

Organic acids and pectins were included with the soluble carbohydrate fraction; 1 mol of each was considered equivalent to .5 and 1 mol of soluble carbohydrate, respectively. No significant improvement in the overall fit of the model was achieved by accounting for these three substrates separately.

The basic metabolic equations and the mathematical method of parameter estimation previously reported were used. An exception was that squared errors were weighted for the number of animals fed a given diet as well as for variance of each product (Koong et al., 1975).

An arbitrary set of initial parameter values for the proportions of each substrate fermented to various VFA and for the microbial growth yield coefficient (1/apparent $Y_{ATP}$) were used as input to begin the iterative processes. In sequential steps, the parameter values were systematically adjusted until weighted sums of squares of residuals (WSSR) converged to a minimum value. Standard errors of the final parameter values for each group were estimated by the jackknife procedure (Miller, 1974).

When nonlinear least-squares methods are used for parameter estimation, there is the possibility that the solution set obtained is not unique. This problem was examined by fixing one parameter in the roughage group, the proportion of a particular substrate being fermented to propionate, away from the value deduced by the program. The remaining 15 parameters were allowed to vary during iteration in an attempt to derive another set that would fit the data as well as or better than, the starting set. This procedure was repeated for each of the five substrates used as model input.

Residuals, i.e., observed minus predicted molar proportions of VFA, were examined in a manner similar to that suggested by Draper and Smith (1966).
Results and Discussion

Deduced fermentation parameters for both data groups, along with estimated standard errors, are presented in Table 1. Soluble carbohydrate and starch showed the largest differences when roughage and concentrate fermentation parameters were compared. This would be expected, because dramatic changes in rumen microbial population have been shown to accompany adaptation to large proportions of readily fermentable carbohydrate in the diet (Mackie et al., 1978). In particular, recent evidence suggests that the increased proportion of fermented starch metabolized to propionate results from an adaptive balance between amylolytic and lactate-utilizing bacteria with high concentrate diets (Mackie and Gilchrist, 1979).

It is also indicated that differences exist in the fermentation parameters for hemicellulose and cellulose. This observation supports their inclusion as separate substrates in the model. A combined digestible cell wall fraction does not appear capable of explaining differences in overall fermentation pattern among diets.

On the basis of metabolic equations used in the model, parameters from Table 1 can be expressed as molar percentages of each VFA formed during fermentation of a particular substrate. The indicated fermentation patterns were then comparable with those estimated in other studies. Results of this exercise are shown in Table 2 for soluble carbohydrate, hemicellulose and cellulose.

Experiments by Wallnofer et al. (1966) with [U-14C] glucose yielded a fermentation pattern for soluble carbohydrate that agrees favorably with that indicated for concentrate diets. Larger amounts of organic acids and pectins in forages, here included with soluble carbohydrate, could account for some of the discrepancy noted between their data on alfalfa hay and those on the roughage diets examined here.

In vitro studies of Sutton (1968) involved small quantities of unlabeled carbohydrate added during incubation to 150 g of rumen contents. His results showed the effect of added sugar on overall fermentation pattern and not on the pattern for an individual substrate. It was not surprising, therefore, that these data differ from those from other studies.

Studies with isolated [14C] hemicellulose from perennial ryegrass (Bath and Head, 1961) and orchardgrass (Satter et al., 1964) yielded fermentation patterns quite close to that indicated for hemicellulose by our method. Isolated and computer estimated hemicellulose fermentation patterns, however, are not consistent with those obtained with [1-14C] xylose (Wallnofer et al., 1966).

Cellulose appeared to undergo fermentation in a fashion very different from that estimated previously. A much higher molar percentage of

<table>
<thead>
<tr>
<th>TABLE 1. ESTIMATED FERMENTATION PARAMETERSa</th>
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<tr>
<td>Substrate</td>
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<tr>
<td>Soluble carbohydrate</td>
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<tr>
<td></td>
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<tr>
<td>Starch</td>
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<td></td>
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<tr>
<td>Hemicellulose</td>
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<td></td>
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<tr>
<td>Cellulose</td>
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<td>Protein</td>
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aDesignations of Koong et al. (1975) for the proportion of fermented substrate converted to each product.

bR = roughage group, mean ± SE; n = 10.

cC = concentrate group, mean ± SE; n = 8.
TABLE 2. COMPARISON WITH PREVIOUS IN VITRO ESTIMATES OF SUBSTRATE FERMENTATION

<table>
<thead>
<tr>
<th>Diet/reference</th>
<th>Soluble carbohydrate</th>
<th>Hemicellulose</th>
<th>Cellulose</th>
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<tbody>
<tr>
<td></td>
<td>A^a</td>
<td>P</td>
<td>B</td>
</tr>
<tr>
<td>Roughage group</td>
<td>73.0</td>
<td>21.7</td>
<td>5.3</td>
</tr>
<tr>
<td>Alfalfa hay^b</td>
<td>62.8</td>
<td>12.1</td>
<td>(25.1)^c</td>
</tr>
<tr>
<td>Dried grass^d</td>
<td>63.2</td>
<td>26.0</td>
<td>9.8</td>
</tr>
<tr>
<td>Hay^e</td>
<td>69.3</td>
<td>23.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Concentrate group</td>
<td>54.2</td>
<td>25.3</td>
<td>18.1</td>
</tr>
<tr>
<td>Concentrate^b</td>
<td>59.0</td>
<td>22.3</td>
<td>(18.7)</td>
</tr>
<tr>
<td>70%+ concentrate^e</td>
<td>64.5</td>
<td>27.5</td>
<td>7.4</td>
</tr>
<tr>
<td>80% concentrate^f</td>
<td>45.3</td>
<td>32.2</td>
<td>16.3</td>
</tr>
</tbody>
</table>

^a Acetate, propionate, butyrate, valerate.


^c Approximately 90% butyrate.

^d Bath and Head (1961). 14C-labeled hemicellulose and α-cellulose isolated from perennial ryegrass.

^e Satter et al. (1964). 14C-labeled hemicellulose and α-cellulose isolated from orchardgrass.

^f Sutton (1968). Sucrose; average of xylose and arabinose.
acetate resulting from cellulose degradation is indicated. This type of metabolism is consistent with that of one major rumen cellulolytic species, Ruminococcus albus. In fact, Wolin (1975) found that acetate was the only organic product of glucose fermentation when this organism was cultured with another capable of utilizing hydrogen.

Two points must be considered when the cellulose fermentation data from this study are compared with those of Wallnofer et al. (1966). Less than 10% of their cotton [14C] cellulose was fermented during the 4 h in vitro incubation period, even after soaking overnight in water and 6 h of rumen preincubation for substrate colonization. Secondly, there is evidence to suggest that cellulolytic microbial species differ in their ability to digest more resistant forms of this substrate (Bryant, 1973). Cotton fibers could, therefore, be an inappropriate substrate for estimating the fermentation pattern of usual dietary celluloses.

Data of Bath and Head (1961) and Satter et al. (1964) on isolated [14C] α-cellulose are also difficult to interpret. Both groups of researchers used the procedure described by Bath (1960) to recover cellulose from forage grown under a 14CO2 atmosphere. This method required one or two 30-min treatments with acid chlorite to remove lignin prior to extraction of hemicellulose and cellulose. Corbett (1963) reported that hypochlorite treatment caused considerable degradation of cotton linters. It is doubtful that such harsh isolation procedures yield a cellulose similar to that normally digested by rumen microorganisms.

Apparent YATP estimated for the roughage and concentrate groups were 9.88 and 10.59 ± .07, respectively. The accuracy of these values depends on estimates of microbial production for various diets. Few data of this kind are available, and consequently, microbial production was not included as an error criterion in this study. More emphasis on apparent YATP values will be possible as additional measurements of microbial growth are obtained.

Initial average errors and final weights from the application of fermentation parameters in table 1 to both data groups are presented in table 3. Between-animal variation within diets is commonly 5% for acetate. A level of error has been reached, therefore, beyond which further progress is constrained by normal biological variability. The concentrate data group has an average overall error somewhat greater than that of the roughage group. Further improvement has been made difficult by increased variance associated with concentrate diets, as evidenced by the higher weights for this group shown in table 3. This increased variance with concentrate diets has been observed by others and is of the same magnitude as that reported by Weiss et al. (1967).

Results from the tests of solution uniqueness

| TABLE 3. AVERAGE ERRORS AND FINAL WEIGHTS FOR ESTIMATED FERMENTATION PATTERNS |
|------------------|--------|--------|--------|--------|--------|
| Group            | Acetate| Propionate| Butyrate| Valerate| Overall |
| Roughage         |        |          |         |         |        |
| Initial error, %a (n)b | 3.7 (60) | 3.2 (60) | 2.5(60) | 3.1(40) | 3.1    |
| Corrected error, % | 3.3    | 2.4     | 2.5     | 3.1     | 2.8    |
| Weights          | 13.8   | 10.0    | 5.9     | 8.2     |        |
| Concentrate      |        |          |         |         |        |
| Initial error, % (n) | 4.5 (48) | 5.2 (48) | 2.5 (48) | 2.1 (32) | 3.7    |
| Corrected error, % | 4.2    | 5.1     | 2.5     | 2.1     | 3.6    |
| Weights          | 31.1   | 35.5    | 9.3     | 5.1     |        |

aAverage (observed—predicted) percentage VFA, calculated as:

\[ \frac{1}{n} \sum_{i=1}^{n} \left( \frac{E (obs.-pred.)}{n} \right) \]

bNumber of diets on which average was based.

cEstimated variance of product estimate.
Figure 1. Sum of the weighted squares of residuals (WSQR) as influenced by changes in the proportion of substrate fermented to propionate away from the deduced parameter value ("S" labeled segment). (A) Soluble carbohydrate; (B) Starch; (C) Hemicellulose; (D) Cellulose; (E) Protein.
Rumen fermentation parameters are given in figure 1. In each graph, a series of vertical segments marks the iterative progress in reducing WSQR and the number of steps required between each point. The “S” labeled segment indicates a solution value for the proportion of that substrate fermented to propionate. The horizontal distance between this and other segments indicates the magnitude of the imposed change from the value deduced by the program. Vertical distance between segment minima demonstrates the sensitivity of a parameter to this change. Uniqueness is evidenced when the curve connecting a graph’s segment minima is concave.

Soluble carbohydrate (figure 1A) and starch (figure 1B) both show distinct “U” shaped response curves, suggesting that the minimum error is indeed near the solution value and the deduced parameter values describing their fermentation are unique. Hemicellulose (figure 1C) and cellulose (figure 1D) have broader minima, but their graphs are still concave. The flat response curve for protein (figure 1E) shows that changing the proportion of this substrate fermented to propionate has little effect on overall error. The fermentation parameters estimated for protein, then, are not unique. This is not surprising and results from the fact that protein inputs were similar across diets and, thus, lacked the variation required for deduction of a unique fermentation pattern.

The next step in the analysis was to examine residuals for systematic error. Graphed in figure 2A are acetate residuals vs observed percentage acetate (percentage of an individual VFA was defined as moles of that VFA/100 mol total VFA) for the roughage group. Rather than a random scatter of values, an obvious relationship exists between these variables. Acetate was overestimated at lower values and underestimated at higher ones. The same type of systematic error was noted with propionate (figure 2B).

Lignin plus ash as a percentage of dry matter (X1) was identified as the main cause of systematic error for the roughage group. Regression equations and associated statistics are: Y1 = .45X1 - 6.10, Sb = .12 and r2 = .21 for the acetate residual (Y1) and Y2 = -.49X1 + 6.43, Sb = .08 and r2 = .36 for the propionate residual (Y2). Neither slope was equal to zero (P<.001). It should not be concluded that the systematic error was related to just digestible organic matter [1-(lignin + ash)], since inputs to the model were amounts of the various substrates digested in the rumen per day. This relationship, therefore, suggests an additional influence of lignin plus ash on fermentation pattern.

The coefficients of determination indicate that lignin plus ash explained 21% of the total remaining error variance for acetate and 36% of that remaining for propionate. Remaining variation is mostly random and falls within experimental variance. Another interesting point is that the regression slopes were nearly inverses. This implies that initial overestimates of acetate resulted in a corresponding underestimate of propionate and vice versa.

Concentrate diets exhibited the same systematic error when residuals were graphed...
Figure 3. Concentrate group residuals vs observed mol/100 mol of VFA. (A) Acetate; (B) Propionate.

The agreement between predicted and observed fermentation pattern across all diets is shown in figure 4. In each case, the appropriate statistical test was whether the slope of the regression for each group of data was different from 1. An ideal fit is, therefore, indicated by the solid line with this slope in each graph. Predicted vs observed percentage acetate against observed percentage acetate (figure 3A) and propionate (figure 3B). Percentage roughage in the diet ($X_2$) was the only identifiable source of systematic error for the concentrate group. Regression equations and associated statistics are: $Y_1 = .09X_2 - 1.18, Sb = .04$ and $r^2 = .10$ for the acetate residual ($Y_1$) and $Y_2 = -.07X_2 + 1.14, Sb = .05$ and $r^2 = .05$ for the propionate residual ($Y_2$). The slope of acetate residuals vs percentage roughage was different from zero (P<.05), whereas that of propionate residuals vs percentage roughage was not (P>.05).

The systematic error relationships described above were used to correct estimated fermentation patterns for each diet. The adjustment was based on percentage lignin plus ash for diets in the roughage group, and percentage roughage for those in the concentrate group. Corrected average errors for both data groups are presented in table 3.

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Rumen fermentation parameters

No bias was indicated for predicted vs observed percentage propionate (figure 4B), as neither group exhibited a slope different from 1 (P>.05). Coefficients of determination for propionate in the roughage and concentrate groups, respectively, were .30 and .40.

A bias was again shown (P<.05) in the graph of predicted vs observed percentage butyrate (figure 4C) for the roughage group. The indicated coefficient of variation for the observed percentage butyrate was, however, 22.7%, a factor that probably makes the cause of this bias unidentifiable. Roughage and concentrate groups had coefficients of determination for butyrate of .12 and .14, respectively.

The model was then fitted with the final parameter set determined for the roughage group (table 1) and used to estimate the molar production of various VFA from those diets for which validation data were available. Results are shown in figure 5 for 47 sheep and 10 cattle or water buffalo. Extreme accuracy in the prediction of molar VFA production was evidenced by coefficients of determination of .97, .95 and .95 for acetate, propionate and butyrate, respectively. These values remained high for the sheep data alone, .72, .68 and .46, respectively. Not enough data were available to justify separate estimation of the accuracy of the model in predicting VFA production in cattle.

This method has permitted products of fermentation to be estimated with accuracy rivaling that with which they can be measured by current laboratory methods. The results of this study also give further support to the use of analytical models based on theoretical equations in research on ruminant nutrition.

Literature Cited


