Effects of BioChlor and Fermenten on Microbial Protein Synthesis in Continuous Culture Fermenters*

I. J. Lean, T. K. Miller Webster, W. Hoover, W. Chalupa, C. J. Sniffen, E. Evans, E. Block, and A. R. Rabiee

1Bovine Research Australasia, Camden 2570, NSW, Australia
2Rumen Fermentation Profiling Laboratory, Division of Animal and Veterinary Science, West Virginia University, Morgantown 26506
3School of Veterinary Medicine, University of Pennsylvania, Kennett Square 19348
4Fencrest, Holderness, NH 03245
5Essi Evans Technical Advisory Services, Inc., Bowmanville, ON, Canada L1C 3J1
6Church & Dwight Co., Inc., Arm & Hammer Animal Nutrition Group, Princeton, NJ 08543

ABSTRACT

Meta analysis models were constructed from a data-set of 15 continuous culture fermenter trials and 118 observations on studies with either BioChlor (n = 23 observations) or Fermenten (n = 95) included at 10 and 3%, respectively, of dietary dry matter (DM) to evaluate effects of the ingredients BioChlor and Fermenten (B/F) on rumen function. Digestibility of crude protein was significantly increased by 11% with B/F treatment. This was reflected in significant increases in digestibility of DM and organic matter (OM) by 3.6 and 7.9%, respectively. Increased amounts of sugar in the diet in the presence of B/F tended to reduce digestibility of non-structural carbohydrates (NSC); however, the net effect on NSC digestion was small. There was no effect of treatment on most individual volatile fatty acids (VFA) or total VFA production. Propionate production, however, was significantly reduced in treated fermenters. The main effect of B/F as well as of starch and soluble fiber when combined with the treatment was to increase propionate production; however, the interaction between B/F treatment and sugar decreased propionate production markedly, resulting in a net decrease. The acetate-to-propionate ratio increased by 6% with B/F, largely as a result of the decrease in propionate. Production of nonammonia nitrogen was 1% less in B/F-treated fermenters, and interactions and starch, sugar, or soluble fiber were significant. Treated fermenters produced 15.7% more microbial nitrogen, in association with a significant 37% increase in rumen protein digestion. Interactions between treatment and starch, soluble fiber, or sugar influenced these results.

The interaction of B/F and sugar resulted in a decrease in undegradable protein N and an increase in microbial nitrogen production. Ammonia nitrogen concentrations were increased by 24.6% in treated fermenters. Efficiency of microbial nitrogen production from DM, OM, or carbohydrate was significantly increased by B/F. Sugar content increased efficiency of microbial protein production/kg of OM digested or carbohydrate digested in the presence of treatment by >10 times the increase that was attributable to the interaction of treatment with starch. Treatment with B/F reduced moles of VFA produced/kg of microbial nitrogen produced by 16%. This effect was also substantially influenced by interactions between B/F and sugar. If the fermenter results are representative of those in vivo, milk production responses to treatment with B/F will depend on amounts of starch, soluble fiber, and, particularly, sugar in diets. Milk production responses will also depend on the quality of protein in the diet and the comparative benefit that increased flux of microbial nitrogen provides. Increased digestibility of OM should allow additional ruminant production benefits.

(Key words: BioChlor, Fermenten, continuous culture fermentation, rumen microbial protein production)

Abbreviation key: AMN = ammonia nitrogen, B/F = BioChlor and Fermenten, CPD = CP digestibility, NSC = nonstructural carbohydrate.

INTRODUCTION

BioChlor and Fermenten (B/F) are products derived from by-product streams of fermentation. The products contain high concentrations (107 g/kg BioChlor and 82 g/kg Fermenten) of free AA and peptides up to 10 AA in length (T. K. Miller-Webster and W. Hoover, unpublished data, 2003). Peptides and AA provide an ideal substrate for microbial growth and increase the efficiency of microbial protein synthesis (Russell et al., 1992). Results of fermenter studies indicated that treat-
ment with B/F improved microbial nitrogen production by approximately 17% (Chalupa et al., 1997). This response was reflected in feeding studies using BioChlor in lactating cows, where in one trial microbial protein synthesis was increased by 23% and in another by 7%; however, neither increase was significant (Broderick et al., 2000). The objectives of this study were to consolidate and evaluate a large amount of data examining responses of rumen fluid to diets containing B/F and to determine the effects of treatment on digestibility, microbial protein production and efficiency, and VFA production.

Statistical models were developed to assess the effects of treatment and diet composition on fermentation outputs. Factors selected for inclusion in models reflected the chemical structure of the feeds and included CP, solubility of CP, sugar, starch, soluble fiber, and NDF. Interactions between treatment and various dietary components were assessed with the intention of identifying dietary factors that influenced responses.

### MATERIALS AND METHODS

Dairy rations were formulated to support either 45 kg/d of milk production (12 trials) or to meet the needs of transition dry cows 2 wk prior to calving. (Trials 7, 8, and 9 are BioChlor studies.) Mean formulations for trials are presented in Tables 1 and 2. Mean composition of the lactation diets was 53.9% forage, consisting of a mixture of corn silage, mixed haylage, or alfalfa hay, and 46.1% concentrate using corn and barley, soybean meal, and minerals. Fermenten was added at 3% (DM basis) to the treatment lactation rations. Mean composition of the pre-calving rations was 60.1% forage, consisting of corn silage and alfalfa hay, and 39.9% grains, using corn and barley, soybean meal, and minerals. Pre-calving treatment rations received 10% BioChlor (DM basis). Additions of B/F were per manufacturer recommendations. The chemical compositions of BioChlor and Fermenten (percentage DM basis) were, respectively, CP, 52.2, 53.2; soluble protein, 40.5, 41.7; NPN, 30.1, 33.4; acid detergent insoluble protein, 0.47, 0.48; neutral detergent protein, 2.1, 1.6; ADF, 5.6, 5.1; NDF, 21.4, 17.9; physically effective NDF, 1.1, 0.9; lignin, 2.9, 2.5; ash, 7.4, 3.6; ether extract, 3.7, 3.0; NFC, 17.4, 24.0; sugar, 7.1, 7.0; starch 10.3, 17; Ca, 0.9, 0.1; P, 0.8, 0.7; Mg, 0.3, 0.3; K, 1.1, 0.9; S, 2.4, 6.9; Na, 1.2, 0.6; and Cl, 10.1, 0.6.

Fifteen trials involving B/F conducted from 2000 to 2004 were evaluated for consistency of approach. Treatments that were not controlled by formulation when Fermenten was added were excluded from the data. For example, if a potentially competing or complimentary product was used in a trial for evaluation purposes, these fermenter results were excluded (3 fermentation evaluations in this case). A study that did not have control groups was excluded (one study). When treatments were matched within a group of fermenter studies, these were treated as separate trials. After preliminary statistical evaluation, BioChlor responses were not found to differ statistically from those of Fermenten, and, more importantly, there were no major differences in point effects of these treatments on outcomes. Consequently, BioChlor studies were not treated differently from Fermenten studies to provide a data set with 15 trial groups and 118 observations. Twenty-three cases in this data set were derived from BioChlor studies.

### Table 1. Descriptive statistics of diet inputs including CP, Soluble CP, NDF, and nonstructural carbohydrates (NSC) for all herds included in the model.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>18.10 ± 0.0</td>
<td>18.0 ± 0.11</td>
<td>24.80 ± 0.0</td>
<td>31.15 ± 0.90</td>
<td>28.80 ± 0.0</td>
<td>29.95 ± 0.16</td>
</tr>
<tr>
<td>Soluble CP</td>
<td>17.74 ± 0.21</td>
<td>17.92 ± 0.00</td>
<td>20.59 ± 3.3</td>
<td>23.04 ± 0.00</td>
<td>33.80 ± 0.11</td>
<td>32.90 ± 0.00</td>
</tr>
<tr>
<td>NDF</td>
<td>17.20 ± 0.00</td>
<td>17.20 ± 0.00</td>
<td>29.30 ± 0.00</td>
<td>35.40 ± 0.00</td>
<td>32.80 ± 0.00</td>
<td>32.20 ± 0.00</td>
</tr>
<tr>
<td>NSC</td>
<td>18.30 ± 0.00</td>
<td>18.50 ± 0.00</td>
<td>22.70 ± 0.00</td>
<td>24.20 ± 0.00</td>
<td>34.20 ± 0.00</td>
<td>35.30 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 5</td>
<td>17.30 ± 0.00</td>
<td>17.80 ± 0.00</td>
<td>31.73 ± 0.00</td>
<td>26.40 ± 0.00</td>
<td>32.00 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 6</td>
<td>18.90 ± 0.33</td>
<td>18.70 ± 0.00</td>
<td>30.40 ± 1.20</td>
<td>35.30 ± 7.6</td>
<td>33.10 ± 0.11</td>
</tr>
<tr>
<td>±</td>
<td>Trial 7</td>
<td>15.44 ± 0.00</td>
<td>15.22 ± 0.16</td>
<td>26.94 ± 0.00</td>
<td>52.08 ± 0.5</td>
<td>39.57 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 8</td>
<td>16.82 ± 0.00</td>
<td>17.29 ± 0.00</td>
<td>19.86 ± 0.00</td>
<td>40.31 ± 0.00</td>
<td>32.40 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 9</td>
<td>15.05 ± 0.00</td>
<td>14.90 ± 0.00</td>
<td>28.22 ± 0.00</td>
<td>46.41 ± 0.00</td>
<td>31.90 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 10</td>
<td>17.70 ± 0.00</td>
<td>18.00 ± 0.00</td>
<td>25.00 ± 0.00</td>
<td>26.50 ± 0.00</td>
<td>35.60 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 11</td>
<td>17.80 ± 0.00</td>
<td>18.10 ± 0.00</td>
<td>26.37 ± 0.00</td>
<td>28.50 ± 0.00</td>
<td>32.80 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 12</td>
<td>17.90 ± 0.00</td>
<td>17.90 ± 0.00</td>
<td>25.63 ± 0.00</td>
<td>28.50 ± 0.00</td>
<td>31.80 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 13</td>
<td>18.40 ± 0.00</td>
<td>18.20 ± 0.00</td>
<td>35.10 ± 0.00</td>
<td>39.70 ± 0.00</td>
<td>30.20 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 14</td>
<td>18.20 ± 0.00</td>
<td>17.80 ± 0.00</td>
<td>35.00 ± 0.00</td>
<td>37.40 ± 0.00</td>
<td>32.10 ± 0.00</td>
</tr>
<tr>
<td>±</td>
<td>Trial 15</td>
<td>18.80 ± 0.00</td>
<td>19.00 ± 0.00</td>
<td>27.80 ± 0.00</td>
<td>28.60 ± 0.00</td>
<td>35.60 ± 0.00</td>
</tr>
</tbody>
</table>

1BioChlor (registered trademark of Church & Dwight Co., Inc. Princeton, Nj) studies.
Continuous Culture System

A 12-unit continuous culture system similar to that described by Stern and Hoover (1990) was used. Each fermenter had a working volume of 1164 mL, and all diets were fermented in triplicate under the following conditions. Lactating rations had a liquid dilution rate of 13%/h and a solids retention time of 22 h; pre-calving rations had a liquid dilution rate of 10%/h and a solids retention time of 27 h. Feed intakes per 24 h were 100 g DM and 60 g DM for the lactating and pre-calving rations, respectively. Incubation temperatures were maintained at 39°C across all fermentations. The fermentation pH was not controlled but was monitored daily. Inocula for the fermenters were obtained from 2 ruminally cannulated, lactating or dry Holstein cows. Rumen fluid was pooled before inoculating the fermenters. Diets were fed automatically in 2 equal feedings at 12-h intervals or in 4 equal feedings at 6-h intervals, for the pre-calving and lactating rations, respectively.

The artificial saliva of Weller and Pilgrim (1974) was continuously infused at a rate to provide the liquid flow for fermentation periods of 10 d. The first 7 d were for equilibration. During the last 3 d, the effluents were collected in an ice bath, and a 1-L sample was composited and saved for analysis.

After the effluent was collected on d 10, contents of fermenters were allowed to settle, and the fluid layer was used for collection of microbes. Two 200-mL samples were taken from each fermenter and centrifuged for 20 min at 200 × g. The supernatants were centrifuged for 15 min at 30,000 × g, the pellets combined, re-suspended in saline, and again centrifuged for 15 min at 30,000 × g. The supernatants were discarded, and the pellets were resuspended in 20 mL of a 50:50 mixture of distilled water and methanol and centrifuged for 15 min at 30,000 × g. The supernatants were poured off, and the pellets resuspended in distilled water and lyophilized.

Chemical Analyses

The feed DM was determined by oven drying at 100°C for 24 h. Effluent DM was determined by centrifuging a 34- to 40-g sample of effluent at 30,000 × g for 45 min. The supernatant was discarded, and the particulate matter was dried at 110°C for 24 h and reweighed. Determination of the NDF and ADF content in the feed was according to the methods of Goering and Van Soest (1970) with modifications by Van Soest et al. (1991). Total N in feed, effluents, bacterial N, and ammonia N was determined according to AOAC (1990) using an automated Tecator digestion system (Tecator, Inc., Herndon, VA). Ether extraction of the feed was performed according to AOAC (1990). Analysis of VFA was performed in accordance with the GLC separation procedure described in the literature (Anonymous, 1975). Effluent and bacterial concentrations of purines were determined by the procedures of Zinn and Owens (1986). Starch was measured in the feeds and effluents by an enzymatic method (Solvay Enzymes, 1996), and glucose concentration was measured using glucose oxidase as described by Karkalas (1985). The sugars (water-soluble carbohydrates) of the feeds and effluents were determined by the method described in Hall et al. (1999).

Statistical Analysis

Data were evaluated by univariate means to examine relationships, normality, and missing data. Subse-
Fermentation by-products on microbial protein synthesis frequently, after exploratory multivariate analyses, data were evaluated using SPSS V 11.5 GLM, with trial as a random effect.

Covariates selected for inclusion in models reflect the composition and chemical analysis of feeds and included CP, solubility of CP, sugar, starch, and NDF. Soluble fiber was calculated from the difference between nonstructural carbohydrate (NSC) and NFC and was also included as a covariate. Lastly, 2-way interactions between treatments were examined. Interactions between treatments and NDF content of the diet were nonsignificant. Propionate and isobutyrate concentrations were log-transformed, and isovalerate levels were square root-transformed for analysis. Models that included interactions between B/F combined treatment and sugar, starch, and soluble fiber are reported.

Models took the form

\[ u = \text{Intercept} + \text{TREAT} + \text{TRIAL} + \text{CP} + \text{SOLCP} + \text{NDF} + \text{STARCH} + \text{SUGAR} + \text{SOLFIB} + (\text{TREAT} \times \text{STARCH}) + (\text{TREAT} \times \text{SUGAR}) + (\text{TREAT} \times \text{SOLFIB}) + \text{error} \]

where

- \text{TREAT} = \text{fixed effect of B/F},
- \text{TRIAL} = \text{random effect of trial},
- \text{CP} = \text{fixed effect of CP in the diet},
- \text{SOLCP} = \text{fixed effect of soluble CP in the diet},
- \text{NDF} = \text{fixed effect of NDF in the diet},
- \text{STARCH} = \text{fixed effect of starch in the diet},
- \text{SUGAR} = \text{fixed effect of sugar in the diet},
- \text{SOLFIB} = \text{fixed effect of soluble fiber in the diet},
- \text{TREAT} \times \text{STARCH} = \text{interaction of treatment (control or B/F) with starch in the diet},
- \text{TREAT} \times \text{SUGAR} = \text{interaction of treatment (control or B/F) with sugar in the diet},
- \text{TREAT} \times \text{SOLFIB} = \text{interaction of treatment (control or B/F) with soluble fiber in the diet}

Residual analysis and goodness of fit statistics were used to assess models, and models included had good fit and a good pattern of residuals. Finally, more basic statistical models that had nonsignificant terms removed were used to examine the point directions and magnitude of coefficients for significant interaction terms; these are reported in Tables 3 to 6. Factors were treated as significant at a \( P \) value of <0.05, but \( P \) values of <0.1 are reported.

RESULTS

Examination of the mean formulations used in the studies (Tables 1 and 2) shows that there was a wide range of dietary inputs, reflecting both pre-calving and lactating cow diet formulations. Trials were conducted over 4 yr, during which nutrient composition of the diets reflected changes in feedstuffs and forages available for formulation.

Effects of Treatments on Digestibility of CP, DM, OM, NDF, NSC, and Total Carbohydrate

Digestibility of CP (CPD) was increased 11% by B/F treatment \((P = 0.001; \text{Table 3}; \text{Figure 1})\). In the CPD model evaluating potential interactions, the interaction terms of treatment with starch and sugar were significant, as was the effect of treatment (Table 3). Starch, and in particular sugar, increased CPD in the presence of treatment.

Digestibility of DM and OM (Figure 2) was increased by treatment (Table 3) by 3.6 and 7.9%, respectively. Interactions of treatment with the NFC components of the diet were not significant in these models.

The B/F treatment did not significantly affect digestibility of NDF or total carbohydrates \((P > 0.5; \text{Table 3})\).

For NSC digestibility, the final model included terms for treatment \((P = 0.004)\) and the interaction between treatment and sugar \((P = 0.01)\). Increasing sugar in the diet reduced the positive effect of treatment on NSC digestibility, and mean NSC digestibilities were very similar for treatment groups (Table 3).

Prediction of pH and VFA

Very few variables significantly predicted \( \text{pH} \) and total or individual \( \text{VFA} \) (Table 4). However, propionate and the ratio of acetate to propionate were significantly influenced by treatment, and the effect of treatment on valerate concentrations approached significance \((P = 0.061)\).

Propionate production was significantly lower for the B/F-treated fermenters \((P = 0.001)\). All interactions between treatment and NFC and the main effect of treatment were significant. The main effect of treatment was to decrease propionate; interactions between treatment and starch or soluble fiber were positive, although less than contributed in control fermenters. The interaction of treatment with sugar was negative, substantially resulting in a net negative effect of treatment.

Further evidence for a shift in \( \text{VFA} \) production with treatment was provided by the acetate-to-propionate ratio, which significantly increased by 6% with treatment \((P = 0.001)\). Interactions between starch, sugar, or soluble fiber with treatment were significant.
Table 3. Effect of BioChlor and Fermenten1 (B/F) on digestibilities of diets. Final models, excluding nonsignificant terms, significance of terms, and coefficients in the model.

<table>
<thead>
<tr>
<th>Coefficients for interactions of treatment with components of nonstructural carbohydrates (NSC) are reported for the control group and treatment group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein and carbohydrate Soluble Soluble Treatment × digestibility Control B/F Treatment Trial CP CP fiber starch sugar3</td>
</tr>
<tr>
<td>[mean ± SE; (coefficient)] P value; (coefficient)</td>
</tr>
<tr>
<td>CP digestibility, %</td>
</tr>
<tr>
<td>DM digestibility, %</td>
</tr>
<tr>
<td>OM digestibility, %</td>
</tr>
<tr>
<td>NDF digestibility, %</td>
</tr>
<tr>
<td>NSC digestibility, %</td>
</tr>
<tr>
<td>Total carbohydrate digestibility, %</td>
</tr>
</tbody>
</table>

Figure 1. Estimated marginal means of CP digestibility (CPD) following control for covariates for the 15 trials. Trials 7, 8, and 9 were based on BioChlor (Church & Dwight Co., Princeton, NJ) inclusion.

Figure 2. Estimated marginal means of OM digestibility (OMD) following control for covariates for the 15 trials. Trials 7, 8, and 9 were based on BioChlor (Church & Dwight Co., Princeton, NJ) inclusion.

Mortality on Fermenter Studies

Effects of Treatment on Nitrogen Metabolism in Fermenter Studies

The B/F treatment had a significant effect on non-ammonia nitrogen (NAN; g/d) (microbial nitrogen + RUP).

- Treatment control or B/F and starch interaction.
- Treatment control or B/F and sugar interaction.

Permenten and BioChlor are registered trademarks of Church & Dwight Co., Inc. Princeton, NJ.
Table 4. Effect of BioChlor and Fermenten¹ (B/F) on pH and VFA production (mmol/d) from diets. Final models, excluding nonsignificant terms.²

<table>
<thead>
<tr>
<th>pH and VFA</th>
<th>Control</th>
<th>B/F</th>
<th>Treatment</th>
<th>Trial</th>
<th>CP</th>
<th>Soluble CP</th>
<th>NDF</th>
<th>Starch</th>
<th>Soluble fiber</th>
<th>Treatment × starch³</th>
<th>Treatment × sugar⁴</th>
<th>Treatment × soluble fiber⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.253 ± 0.017</td>
<td>6.281 ± 0.015</td>
<td>NS</td>
<td>0.001</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Total VFA production, mmol/d</td>
<td>375.769 ± 4.328</td>
<td>374.555 ± 3.584</td>
<td>NS</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Acetate, mmol/d</td>
<td>228.546 ± 1.908</td>
<td>232.500 ± 1.909</td>
<td>NS (P = 0.142)</td>
<td>0.001</td>
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<tr>
<td>Propionate, mmol/d⁶</td>
<td>4.326 ± 0.025</td>
<td>4.273 ± 0.020</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>Butyrate, mmol/d</td>
<td>36.436 ± 1.000</td>
<td>36.981 ± 0.900</td>
<td>NS</td>
<td>0.001</td>
<td></td>
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</tr>
<tr>
<td>Isobutyrate, mmol/d⁶</td>
<td>0.694 ± 0.012</td>
<td>0.702 ± 0.010</td>
<td>NS</td>
<td>0.001</td>
<td>0.087</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.02; (0.069 and 0.138)⁷</td>
<td>0.010; (0.012 and 0.084)⁷</td>
<td>0.001; (0.038 and 0.041)⁷</td>
</tr>
<tr>
<td>Valerate, mmol/d</td>
<td>10.230 ± 0.422</td>
<td>9.111 ± 0.422</td>
<td>0.061</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Isovalerate, mmol/d⁸</td>
<td>2.214 ± 0.820</td>
<td>2.159 ± 0.074</td>
<td>NS</td>
<td>0.001</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Acetate-to-propionate ratio</td>
<td>3.089 ± 0.081</td>
<td>3.276 ± 0.065</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02; (-0.150 and 0.045)⁷</td>
<td>0.010; (-0.408 and 0.289)⁷</td>
<td>0.001; (-0.247 and -0.108)⁷</td>
</tr>
</tbody>
</table>

¹Fermenten and BioChlor are registered trademarks of Church & Dwight Co., Inc. Princeton, NJ.
²Final model selected on basis of residuals and goodness of fit.
³Treatment (control or B/F) and starch interaction.
⁴Treatment (control or B/F) and sugar interaction.
⁵Treatment (control or B/F) and soluble fiber interaction.
⁶Log transformed.
⁷Coefficients are given for B/F and control, respectively.
⁸Square root transformed.
nitrogen) in the fermenters \((P = 0.016)\). Table 5 shows the means for the controls and B/F groups, demonstrating a slight decrease in NAN flow in B/F-treated fermenters. Interactions between treatments and sugar, starch, or soluble fiber were included in the model, and these interactions with B/F were positive, but did not increase NAN as much as in control fermenters.

Treatment with B/F had a highly significant effect on ammonia nitrogen (AMN) in the fermenters \((P = 0.001)\). Table 5 shows the means for the controls and B/F groups. There was a 24.6% increase in AMN in B/F fermenters (Figure 3). The interaction of sugar, starch, and soluble fiber with B/F resulted in a reduction in the amount of AMN, but the concentration remained higher than that of the controls.

Treatment with B/F significantly decreased the amount of protein bypassing the fermenter by 37% \((P = 0.001)\) (Figure 4). Interactions between B/F and sugar, starch, or soluble fiber were significant, and the main effect of B/F was significant. Interactions of starch and soluble fiber with B/F tended to increase the amount of bypass protein, but the interaction with sugar content of the diet markedly decreased the bypass protein output (Table 5).

Treatment with B/F significantly increased the grams of microbial nitrogen produced \((P = 0.001)\) by 15.7% (Figure 5). Interactions between B/F and sugar and starch were highly significant, and the interaction with soluble fiber approached significance \((P = 0.089)\). The interaction between B/F with starch, and especially sugar, in the diet increased microbial nitrogen production. The effect of the interaction between B/F and soluble fiber was to decrease microbial protein nitrogen production (Table 5).

The B/F treatment significantly increased microbial protein nitrogen \( \text{g produced/g of DM digested;} \ P = 0.001 \) by 9.9%. Interactions of B/F with starch and sugar were significant and had a profound effect on microbial protein nitrogen \( \text{g produced/g of DM digested;} \). Crude protein content also increased microbial protein produced, and the interaction of starch and B/F had a small negative effect on microbial nitrogen production/g of DM digested (Table 6).

Treatment with B/F significantly increased microbial protein nitrogen \( \text{g produced/kg of carbohydrate digested;} \) by 13.5% \((P = 0.001; \text{Figure 6})\). Interactions of B/F with starch and sugar were significant.

### Table 5. Effect of BioChlor and Fermenten1 (B/F) on nitrogen metabolism of diets. Final models, excluding non-significant terms.

<table>
<thead>
<tr>
<th>Protein nitrogen</th>
<th>Control</th>
<th>B/F</th>
<th>Treatment x starch</th>
<th>Treatment x sugar</th>
<th>Treatment x soluble fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonammonia nitrogen, g/d</td>
<td>2.747 ± 0.008</td>
<td>2.372 ± 0.007</td>
<td>0.016</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Ammonia nitrogen, mg/dL</td>
<td>5.184 ± 0.215</td>
<td>6.59 ± 0.174</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Bypass protein nitrogen, g/d</td>
<td>1.003 ± 0.037</td>
<td>0.732 ± 0.029</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Microbial protein nitrogen, g/d</td>
<td>1.729 ± 0.035</td>
<td>2.00 ± 0.030</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1Fermenten and BioChlor are registered trademarks of Church & Dwight Co., Inc. Princeton, NJ.

2Treatment (control or B/F) and starch interaction.

3Treatment (control or B/F) and sugar interaction.

4Treatment (control or B/F) and soluble fiber interaction.

5Coefficients are given for B/F and control, respectively.
cant, as was the main effect of B/F. Interactions of B/F with starch and sugar in the diet were positive on microbial nitrogen production. The interaction of B/F with sugar acted to markedly increase microbial production from digested carbohydrate (Table 6).

The B/F treatment did not significantly influence ($P = 0.453$) the conversion of feed N to microbial N. However, when terms were included to account for interactions between B/F and the NFC fractions, significant interactions were found between B/F and sugar and soluble fiber concentrations in the diet (Table 6). The negative main effect of B/F on digested feed N converted to microbial N was modified by the interaction of B/F with sugar and with soluble fiber, which increased the percentage of feed N converted to microbial N.

The results of models assessing moles of VFA produced/kg of carbohydrate digested indicated that there was no significant effect of B/F (Table 6). The B/F treatment lowered the moles of VFA produced/kg of microbial N produced ($P = 0.001$) by 16% (Figure 7), resulting in an increase in the coupling of the fermentation. There were significant interactions between B/F and NFC fractions on moles of VFA produced/kg microbial N produced. The interactions of B/F with the NFC fractions were to reduce the VFA produced/kg of microbial N produced.

**DISCUSSION**

Data were evaluated from a series of studies examining the effects of B/F included at 10 and 3% of the diet, respectively, on rumen fermentation conducted in vitro. Diets that were not considered to be controlled were excluded, and treatments that were confounded by other treatments were also excluded. The studies had a wide range of dietary inputs (Tables 1 and 2). There was little difference in final models based on B/F or Fermenten alone, and the combined data provide more statistical power and probably the best insights into the effects of B/F treatment.
Table 6. Effect of BioChlor and Fermenten\(^1\) (B/F) on efficiency of metabolism of diets. Final models, excluding nonsignificant terms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>B/F</th>
<th>Treatment</th>
<th>Trial</th>
<th>CP</th>
<th>Treatment × starch(^2)</th>
<th>Treatment × sugar(^3)</th>
<th>Treatment × soluble fiber(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial protein nitrogen, g produced/kg DM digested</td>
<td>27.346 ± 0.520 (35.677)</td>
<td>30.060 ± 0.435</td>
<td>0.001</td>
<td>0.001</td>
<td>0.048</td>
<td>0.013; (−0.831 and −0.118)(^5)</td>
<td>0.001; (0.659 and 5.595)</td>
<td></td>
</tr>
<tr>
<td>Microbial protein nitrogen, g produced/kg OM digested</td>
<td>41.711 ± 0.702 (45.866)</td>
<td>44.445 ± 0.588 (0)</td>
<td>0.002</td>
<td>0.001</td>
<td>0.003; (−0.512 and 0.622)</td>
<td>0.001; (4.039 and 8.491)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial protein nitrogen, g produced/kg carbohydrate digested</td>
<td>46.808 ± 1.104 (82.990)</td>
<td>53.115 ± 0.931 (0)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001; (−2.777 and −0.797)</td>
<td>0.004; (0.802 and 9.786)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage (digested) feed nitrogen converted to μg microbial nitrogen</td>
<td>90.780 ± 0.418</td>
<td>89.593 ± 0.332</td>
<td>0.453</td>
<td>0.001</td>
<td>0.098; (0.894 and 1.104)</td>
<td>0.001; (5.505 and 5.913)</td>
<td>0.019; (0.844 and 0.928)</td>
<td></td>
</tr>
<tr>
<td>Moles of VFA produced/kg carbohydrate digested</td>
<td>10.149 ± 0.101</td>
<td>9.961 ± 0.101</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moles of VFA produced/kg microbial nitrogen produced</td>
<td>223.163 ± 5.621 (−647.756)</td>
<td>192.268 ± 4.131 (0)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001; (18.979 and 3.222)</td>
<td>0.001; (5.031 and −50.294)</td>
<td>0.024; (13.235 and 9.726)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Fermenten and BioChlor are registered trademarks of Church & Dwight Co., Inc. Princeton, NJ.

\(^2\)Treatment (control or B/F) and starch interaction.

\(^3\)Treatment (control or B/F) and sugar interaction.

\(^4\)Treatment (control or B/F) and soluble fiber interaction.

\(^5\)Coefficients are given for B/F and control, respectively.
Statistical analyses were conducted using random effects models that controlled for dietary CP, NDF, soluble fiber, sugar, starch, solubility of protein of diets, and interactions between B/F and sugar, starch, or soluble fiber in the diet. Random effects models are the models of choice, as these are more conservative than fixed effects models and reflect analyses that may be generalized. Consistency between random effects models and models with trial as a fixed effect (data not presented) was high.

From the initial models, more basic models that included only significant terms were used to examine the effects of treatment. The interaction terms were included in statistical models on the basis of known biological interactions between peptides and the components of NFC, that is, starch, sugar, and soluble fiber. The calculation of soluble fiber, by the difference method, will also include organic acids, such as malic, in the NFC fraction. Given the diets fed, concentrations of organic acids should be small. These interaction terms also help identify optimal dietary strategies for use of B/F. From Table 3 for example, the difference in NSC digestibility between B/F and control groups can be calculated using the mean values from Trial 1 (Tables 1 and 2). For the control, the equation is $-30.83 + 0.66 \times 32.1 \text{ (control} \times \text{starch)} + 0.60 \times 3.7 \text{ (control} \times \text{sugar)} = -6.9$. For the B/F treatment group, the equation is $0.085 \times 32.1 \text{ (B/F} \times \text{Starch)} - 3.42 \times 3.7 \text{ (B/F} \times \text{sugar)} = -9.9$; a 3% difference. The intercept for the model was 81.7 and can be added to both equations, showing that NSC digestibility was 74.8 for controls and 71.8 for B/F. Intercepts were not provided for the models, but are available upon request.

Effects of B/F treatments on protein digestibility were highly significant and increased the digestibility of the protein by 11% (Figure 1). This result is independent of protein in the diet, as CP content of the diet was not a significant covariate and the solubility of the protein was controlled in the analysis. The B/F effect was enhanced by the presence of starch and sugar in the diet. The interaction of B/F with sugar had nearly 10 times the effect on CPD that the interaction of B/F with starch had. Digestibility of DM or OM was increased with B/F by 3.6 and 7.7% (Figure 2), respectively. Digestibility of DM was significantly increased by increased CP content of the diet and tended to be influenced by the soluble fiber content ($P = 0.052$), whereas only B/F treatment and the random effect of trial were significant for OM digestibility. The digestibility of the NDF fraction of the diet was not influenced by B/F, and the net effect of B/F was a very small reduction in digestibility of the NSC fraction of the diet. This decrease was influenced by a positive main effect of B/F treatment, but a substantial reduction in digestibility resulting from an interaction between B/F and sugar (Table 3). Total carbohydrate digestibility was not significantly affected by B/F.

These data provide a consistent pattern showing that B/F significantly improved CP digestibility and, consequently, digestibility of DM and OM. This increase in
Digestibility exceeds the rate of inclusion of B/F in the diet and demonstrates that B/F stimulate rumen fermentation. Significant interactions between B/F and sugar and starch content of the diet in the model predicting protein digestibility show that rapidly available energy substrates, especially sugar, interact with B/F to increase CPD. The improved CPD indicates that protease activity should be increased. Little proteolytic activity can be attributed to protozoa (Bird et al., 1990), and the majority of proteolytic activity is bacterial (Annison, 1956). This increase in CPD may reflect the stimulatory role peptides and AA supplied by B/F in the diet on growth-important proteolytic bacterial species such as Peptostreptococcus (Nocek and Russell, 1988), although many rumen microbes have proteolytic activity (Baldwin and Allison, 1983). It also appears likely, given the complete degradation of the B1 protein fraction and lack of change in NDF digestibility, that degradation of the B2 fraction of the protein is increased, rather than change in the NDF associated B3 fraction of the protein. This improved digestibility of CP, and consequently of DM and OM, indicates the potential for B/F treatment to increase digesta flow rates, feed intake, and production.

Treatment with B/F lowered production of propionate and increased the acetate to propionate ratio by 6%. There was no significant effect on total VFA production or other specific VFA or iso-acids produced, although a decrease in production of valerate approached significance. These results are consistent with the limited effect of B/F on carbohydrate digestion. The significant interaction between the sugar fractions and B/F for both propionate and the acetate-to-propionate ratio provided evidence that the interaction of B/F with sugars in the diet lowered propionate production. Differences in digestibility of carbohydrate fractions that would explain the shift in VFA metabolism were not detected, and the change in fermentation observed probably reflects lower production of propionate arising from increased efficiency of microbial growth. Bacteria that ferment NFC would have responded to the presence of peptides, whereas peptides in growth, whereas peptides are not a source generally used by fiber-fermenting bacteria (Russell and Sniffen, 1987). The same NFC bacteria can have futile cycles, resulting in the spilling of energy when nitrogen sources are limited. Propionate and lactate are produced as a function of maintaining redox balance in the rumen (Wolin, 1975), and NFC-fermenting bacteria Selenomonas ruminantium and Streptococcus bovis switch fermentation end products to propionate and lactate under conditions of rapid growth (Russell and Strobel, 1993). The observed reduction in propionate may reflect more efficient growth of bacteria from incorporation of the peptides into bacterial protein.

Total NAN (bypass plus microbial N) produced in the fermenters was significantly, but not substantially (1%), decreased by B/F. The main effect of B/F was significant in a model including interactions of B/F with starch, sugar, or soluble fiber that acted to increase NAN. There was a significant 24.6% increase in the amount of NAN produced in B/F fermenters that was mainly due to an increase in microbial N (Figure 3), as the amount of nitrogen in the bypass fraction was a significant 37% lower in the B/F fermenters (Figure 4). The effects of the interaction of B/F with starch and with soluble fiber were to increase the bypass fraction, but the interaction with sugar acted to decrease the bypass fraction.

There was a significant 15.7% increase in microbial nitrogen produced by B/F fermenters (Figure 5). These findings indicate that there is a shift in the pattern of metabolism of protein from one in which bypass protein provides more protein N outflow, to one in which microbial protein becomes more dominant. The observed responses to B/F are similar to those observed in fermenter studies of Russell and Sniffen (1987) in which addition of peptides from trypticase increased the efficiency of synthesis of bacterial protein and increased concentrations of extracellular ammonia. Argyle and Baldwin (1989) found a stimulatory effect of peptides on microbial growth of rumen bacteria and that the bacteria grew linearly in response to carbohydrate fermented. The net response to B/F, however, was not an increase in total NAN. Therefore, if the fermenter results are reflected in the field, responses to B/F will depend, to some degree, on the relative quality of the AA provided by the bypass protein fraction of the other feed ingredients as compared with microbial protein produced.

Efficiencies of microbial protein nitrogen production were significantly increased with treatment. Production of microbial nitrogen (g/kg DM digested) was significantly increased by 9.9% with B/F, and microbial protein nitrogen (g produced/g of OM digested) was similarly increased by 6.6%. There was an approximate 13.6% increase in microbial protein nitrogen (g produced/g of carbohydrate digested) (Figure 6). In all 3 cases, the effect of the interaction of B/F treatment with sugar was to increase the efficiency of microbial protein production by 10 times or more the increase observed with B/F treatment with starch or the interaction of the control treatment with sugar (Table 6). Although the interaction of carbohydrate substrates with peptides is well recognized (Russell and Strobel, 1993; Van Kessel and Russell, 1996; Baldwin, 1995), an interaction between the presence of peptides and sugars in the
diet has not been previously identified. The immediacy of availability of both substrates and efficiency of use of both substrates explains, in part, these responses. Stoichiometric advantages solely, however, do not explain the magnitude of difference between use of starch and peptides as a precursor to that of sugar and peptides. This observation suggests that the effect of treatment with B/F may extend beyond the use of peptides.

There was little net effect of B/F on the conversion of digested feed nitrogen into microbial nitrogen. However, the main effect of B/F was to decrease conversion of digested feed nitrogen into microbial nitrogen, but the interactions of B/F with sugar and soluble fiber increased conversion of digested feed nitrogen into microbial nitrogen.

Treatment with B/F markedly lowered the moles of VFA produced/kg of microbial N produced (P = 0.001) by 16% (Figure 7). The interaction of B/F with sugar in reducing the moles of VFA produced/kg of microbial N produced was profound (Table 6). Given that the only significant reduction in a specific VFA was for propionate, this observation supports the previous observation that improved efficiency of microbial growth was associated with lower propionate production. However, there is a possibility that lactic acid production (not measured) might have been increased, an outcome consistent with increased growth of the NFC-fermenting bacteria Selenomonas ruminantium and Streptococcus bovis. Given that pH was not significantly lower in B/F treatments, any increase in lactic acid would have been minor. By contrast, VFA production/kg of carbohydrate fermented was not significantly altered, nor was there a significant or substantial reduction in the efficiency of conversion of feed N to microbial N. These findings provide evidence that the improvements in efficiency of microbial N production did not result from altered efficiency of carbohydrate fermentation overall. The results were probably mediated through changes in digestibility of protein (Table 3) and, consequently, digestibility of DM and OM.

CONCLUSIONS

The results of these studies provide consistent, strong evidence for a mode of action of B/F. The results were robust over a wide range of different diets (Tables 1 and 2) and strongly support the recognized potential for provision of peptides to increase the efficiency of microbial protein production (Russell et al., 1992). The findings also indicate that this action of B/F treatment depends on readily available carbohydrate substrates, especially sugars. There is a need for further research into the role of optimizing these in dietary fractions in diets containing readily available peptides, such as those containing Fermenten and BioChlor. Production responses to B/F treatment in the field will likely depend on the AA profile of the rest of the diet, with most substantial responses observed when the AA content of the diet is deficient or imbalanced. Further, production responses will also depend on increased inclusion of starch, soluble fiber, and especially sugar in the diet. The potential for treatment to improve digestibility of DM and OM in vitro, suggests that DM availability may need to be increased for animals to express responses to treatment fully.

REFERENCES