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RUMEN MICROBIAL DIGESTION AND AMINO ACID FLOW

1 *Interpretive Summary*

2 Environmental and economic sustainability of milk protein production in dairy cattle requires
3 reducing nitrogen intake while maximizing post rumen flow of amino acids. Byproducts from
4 human food production can provide key nutrients for rumen microbial populations. The objective
5 of this study was to evaluate the effects of a commercial fermentation byproduct on omasal flow
6 of amino acids from non-microbial, bacterial, and protozoal flows. Observed digestion
7 parameters were compared against predictions from a mathematical model. Results indicate that
8 fermentation byproduct can be successfully used to increase post rumen flow of amino acids
9 while maintaining high levels of rumen microbial activity.

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19 **Rumen digestion kinetics, microbial yield, and omasal flows of non-microbial, bacterial**
20 **and protozoal amino acids in lactating dairy cattle fed fermentation byproduct or urea as a**
21 **soluble nitrogen source.**

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27 ***Abstract***

28 The objective of this study was to evaluate the effect of a fermentation byproduct on rumen
29 function, microbial yield and composition and flows of nutrients from the rumen in high
30 producing lactating dairy cattle. Eight ruminally cannulated multiparous Holstein cows
31 averaging (mean \pm SD) 60 ± 10 days in milk and 637 ± 38 kg of body weight were randomly
32 assigned to one of two treatment sequences in a switchback design. Treatment diets contained
33 (dry matter basis) 44% corn silage, 13% alfalfa silage, 12% ground corn, and 31% protein
34 premix containing either a control mix of urea and wheat middlings (**CON**) or a commercial
35 fermentation byproduct meal (Fermenten™, Arm and Hammer Animal Nutrition, Princeton, NJ)
36 at 3% diet inclusion rate (**EXP**). The trial consisted of three 28 d experimental periods, where
37 each period consisted of 21 d of diet adaptation and 7 d of data and sample collection. A triple-
38 marker technique and doubly-labeled $^{15}\text{N}^{15}\text{N}$ -urea were used to were used to measure protozoal,
39 bacterial, and non-microbial omasal flow of AA. Rumen pool sizes and omasal flows were used
40 to determine digestion parameters, including fractional rates of carbohydrate digestion, microbial

41 growth, and yield of microbial biomass per gram of degraded substrate. Fermentation byproduct
42 inclusion in EXP diets increased microbial N and amino acid N content in microbes relative to
43 microbes from CON cows fed the urea control. Microbial amino acid profile did not differ
44 between diets. Daily omasal flows of AA were increased in EXP cows as a result of decreased
45 degradation of feed protein. The inclusion of the fermentation byproduct increased non-microbial
46 AA flow in cows fed EXP vs. CON. Average protozoa contribution to microbial N flow was
47 16.8%, yet protozoa accounted for 21% of the microbial AA flow, with a range of 8 to 46% for
48 individual AA. Cows in this study maintained an average rumen pool size of 320 g of microbial
49 N and bacterial and protozoal pools were estimated at 4 different theoretical levels of selective
50 protozoa retention. Fractional growth rate of all microbes was estimated to be 0.069 h^{-1} , with a
51 yield of 0.44 g microbial biomass per g of carbohydrate degraded. Results indicated that
52 fermentation byproduct can increase omasal flow of AA while maintaining adequate rumen N
53 available for microbial growth and protein synthesis. Simulations from a developmental version
54 of the Cornell Net Carbohydrate and Protein System indicated strong agreement between
55 predicted and observed values, with some areas key for improvement in AA flow and bacterial
56 vs. protozoal N partitioning.

57 **Keywords:** rumen protozoa, amino acids, microbial growth, CNCPS, Fermenten

58 INTRODUCTION

59 Byproducts of human food production have successfully been used to improve the
60 sustainability of the dairy industry (VandeHaar and St-Pierre, 2006). Efficient and effective use
61 of byproduct feeds requires adequate knowledge of the fermentation characteristics of the feed
62 (Fessenden and Van Amburgh, 2016). Fermenten™ (Church & Dwight, Inc., Princeton, NJ) is a
63 commercially available fermentation byproduct feed derived from glutamic acid production and

64 contains high amounts of rumen available nitrogen compounds in the form of soluble AA and
65 small peptides (Fessenden, 2016). Lean et al. (2005) reported an increase in microbial protein
66 flow from continuous fermenters fed fermentation byproduct. The authors attributed this increase
67 in microbial biomass to stimulation of microbial protein synthesis by small peptides and AA
68 (Cotta and Russell, 1982). However, experiments on the same byproducts in vivo have not
69 demonstrated consistent production responses (Broderick et al., 2000, Penner et al., 2009). In a
70 companion paper, Fessenden et al. (20XXa) demonstrated that fermentation byproduct decreased
71 dietary protein degradation in the rumen by approximately 15%, indicating a possible sparing
72 effect of degradable protein through and unknown mechanism. The results from the first portion
73 of the study warranted further investigation into possible effects on omasal AA flow, the
74 partition of N flows between microbial and non-microbial fractions, and the effects on microbial
75 growth and digestion parameters.

76 Mathematical models such as the Cornell Net Carbohydrate and Protein system (CNCPS)
77 (Higgs et al., 2015, Van Amburgh et al., 2015) have been successfully used to optimize rumen
78 microbial output and meet animal nutrient requirements while reducing N losses to the
79 environment (Tylutki et al., 2008). A new, dynamic and more mechanistic version of the CNCPS
80 was developed (Higgs, 2014) that describes rumen degradation of substrates with mechanistic
81 representations of growth of bacteria and protozoa and includes interactions among protozoa and
82 bacteria such as predation and intra-ruminal microbial N turnover. Evaluations of this model
83 indicated a strong ability to predict the partitioning between microbial and non-microbial
84 nitrogen flows; however the partitioning between protozoa and bacteria along with individual
85 AA predictions might require further refinement. As with most model development, evaluations

86 of the rumen sub-model with independent data can be helpful for determining areas for
87 improvement.

88 The hypothesis of this study was that the decreased ruminal protein degradation associated
89 with fermentation byproduct inclusion would increase total AA flow at the omasal canal, with
90 limited effects on bacteria and protozoa growth and turnover. This hypothesis was based off the
91 previous findings regarding microbial and non-microbial N flows reported in the companion
92 paper (Fessenden et al., 20XXa). The objectives of this study were to 1) evaluate the effect of
93 urea with wheat midds or commercial fermentation byproduct on omasal flows of non-microbial,
94 bacterial, and protozoal flows of AA, and 2) provide comparisons of model predicted vs.
95 measured values for rumen microbial digestion and growth parameters.

96 **MATERIALS AND METHODS**

97 The experiment was conducted from April to July 2014 at the Cornell University Ruminant
98 Center in Harford, NY. All animals involved in this experiment were cared for according to the
99 guidelines of the Cornell University Animal Care and Use committee. The committee reviewed
100 and approved the experiment and all procedures carried out in the study.

101 ***Animals, Treatments and Experimental Design***

102 Eight ruminally cannulated multiparous Holstein cows averaging (mean \pm SD) 60 ± 10 d in
103 milk and 637 ± 38 kg of body weight were enrolled in the study. All cows were allowed a 3 wk
104 pre-trial acclimation period where animals were managed and housed in a tie-stall and
105 individually fed a common diet. Cattle were then stratified by pre-trial milk production and
106 randomly assigned to one of two treatment sequences in a switchback design with three 28 d
107 periods. Each period contained 21 d for diet adaptation and 7 d of data and sample collection.

108 Treatment diets contained (DM basis) 44% corn silage, 13% alfalfa silage, 12% ground corn, and
109 31% protein premix containing either a control mix of urea and wheat middlings (**CON**) or
110 Fermenten (**EXP**) at 3% inclusion rate in the final diet (Table 1). All cattle received both diets
111 throughout the three experimental periods according to their randomly assigned sequence of
112 either EXP-CON-EXP or CON-EXP-CON (switchback design). Diets were formulated using
113 CNCPS v. 6.5 (Van Amburgh et al., 2015). Full details of the cattle housing, milking, and
114 feeding management are described in Fessenden et al. (20XXa).

115 *Sample Collection and Processing*

116 Digesta flow leaving the rumen was quantified using the omasal sampling technique
117 developed by Huhtanen et al. (1997) and adapted by Reynal and Broderick (2005). A triple
118 marker system using CoEDTA (Udén et al., 1980), YbCl₃ (modified from Siddons et al., 1985),
119 and undegraded aNDFom (**uNDFom**) (Raffrenato et al., 2018) was used to quantify liquid, small
120 particle, and large particle flow at the omasal canal, respectively. Double-labeled urea (¹⁵N¹⁵N-
121 urea, 98% purity, Cambridge Isotope Laboratories Inc., Andover, MA) was infused into the
122 jugular vein for use as a microbial marker following the method used for studies on urea
123 recycling (Lobley et al., 2000). Details on marker preparation and infusion are reported in
124 Fessenden et al. (20XXa).

125 Samples of whole omasal contents were collected from the omasal canal at 2 h intervals
126 during three 8 h sessions: at 16:00, 18:00, 20:00, and 22:00 h on day 24; at 00:00, 02:00, 04:00,
127 and 06:00 h on day 26; and at 08:00, 10:00, 12:00, and 14:00 on day 27. Sampling times were
128 chosen to encompass every 2 h of the average 24 h feeding cycle. During each 8 h session, a 425
129 mL spot sample was obtained at the first 3 time points, while 675 mL were taken at the last time
130 point. Spot samples were split into subsamples of 50 mL (x2), 125 mL, and 200 mL; with an

131 additional 250 mL subsample at the last time point. The 50 mL subsamples were used for a
132 separate study of nutrient flows (Fessenden et al., 20XXa). The 125 mL subsamples were held on
133 ice and combined within session, yielding a 500 mL sample for bacterial isolation. The 200 mL
134 samples were combined within period and stored at -20°C , yielding a 2.4 L composite for
135 digestion phase separation. The additional 250 mL sample obtained at the end of each session
136 was strained through 2 layers of cheesecloth and immediately processed to isolate protozoa
137 (described later).

138 The 2.4 L pooled omasal composites were thawed and separated into omasal large particle
139 (**LP**), small particle (**SP**) and liquid phase (**LQ**) as described in the companion paper (Fessenden
140 et al., 20XXa). All phase samples were freeze dried and either ground through a 1 mm screen on
141 a Wiley mill (LP) or homogenized with a mortar and pestle (SP and LQ) before analysis.
142 Concentrations of Yb, Co, and uNDFom in each phase were used to calculate the concentration
143 of each nutrient in a theoretical entity representing omasal true digesta (**OTD**) (France and
144 Siddons, 1986). As such, the reported flows and concentrations of any given nutrient in OTD is a
145 mathematical calculation based on re-constitution factors determined using the triple marker
146 technique and measured values of the nutrient in LQ, SP, and LP. This mathematical construct is
147 referred to in this paper as OTD. On the last day of each period, rumen contents were evacuated,
148 weighed, mixed, and a representative sample was obtained for pool size determinations and
149 stored at -20°C prior to lyophilization and determination of rumen nutrient pool sizes.

150 The bacterial isolations from each 8 h sampling period were combined within period to yield
151 an omasal bacteria sample for each cow within period. Microbial isolation was performed
152 according to Whitehouse et al. (1994) with modifications. Briefly; whole omasal contents were
153 filtered through 4 layers of cheesecloth and solids were rinsed once with saline, and the filtrate

154 (I) was treated with formalin (0.1% v/v in final solution) and stored at 4 °C. The solids retained
155 on the cheesecloth were incubated for 1 h at 39 °C in a 0.1% methylcellulose solution, mixed for
156 1 min at low speed (Omni Mixer, Omni International, Kennesaw, GA) to detach solids
157 associated bacteria, and held at 4 °C for 24 h. The contents were then squeezed through 4 layers
158 of cheesecloth and the filtrate (II) was treated with formalin (0.1% v/v in final solution). Filtrates
159 I and II were held at 4 °C until the end of the sampling period, then combined and centrifuged at
160 1000 x g for 5 min at 4 °C to remove small feed particles. The supernatant was centrifuged at
161 15,000 x g for 20 min at 4 °C and the bacterial pellet, representing both solid and liquid
162 associated bacteria, was collected and stored at -20 °C until lyophilization and later analysis.

163 Protozoa were isolated from whole omasal contents using the procedure described by Denton
164 et al. (2015) with modifications (Figure 1). Strained omasal fluid (250 mL) was combined 1:1
165 with pre-warmed, anaerobically prepared Simplex type buffer and added to a pre-warmed
166 separatory funnel. Plant particles were removed by aspiration after 1 h of incubation at 39 °C,
167 allowing for removal of 50 mL of fluid to a pre-calibrated 450 mL line on the funnel. Funnel
168 contents were then preserved with formalin (0.1% v/v in final solution) and stored for < 4 d at 4
169 °C. At the end of each sampling period, preserved contents were centrifuged at 1000 x g for 5
170 min at 4 °C, the pellet was re-suspended in saline, and protozoa were isolated on a nylon cloth
171 with a 20 µm pore size (14% open area, Sefar, Buffalo, NY). The protozoa isolate was washed
172 with saline (500 mL) to reduce bacterial contamination. Microscopic inspection of the retained
173 protozoa and filtrate indicated low feed contamination and good recovery of small protozoa.
174 After isolation, protozoa were stored at -20 °C, followed by lyophilization and measurement of
175 DM amount to calculate yield of protozoal DM per L of omasal fluid (Ahvenjärvi et al., 2002).

176 *Sample Analysis*

177 Samples of freeze-dried bacteria, protozoa, omasal fractions and rumen contents were
178 analyzed for residual DM after 6 h at 105 °C and ash according to AOAC (2005). Total N was
179 determined using a combustion assay (Leco FP-528 N Analyzer, Leco Corp., St. Joseph, MI).
180 Samples were analyzed for non-ammonia nitrogen (NAN) and ¹⁵N as follows: 20 µg of N from
181 each sample was weighed into tin capsules and 10 µL of 72 mM K₂CO₃ were added and
182 incubated at 60°C overnight to volatilize ammonia. Samples were then analyzed for NAN and
183 ¹⁵N using a Carlo Erba NC2500 elemental analyzer interfaced with an isotope ratio mass
184 spectrometer (Cornell University Stable Isotope Laboratory, Ithaca, NY).

185 Amino acid content of bacteria, protozoa, and omasal fractions was determined by HPLC.
186 For all AA excluding Met, Cys, and Trp, sample containing 2 mg N was weighed into hydrolysis
187 tubes with 25 µL of 250 mM Norleucine as an internal standard. Samples were then hydrolyzed
188 at 110 °C for 21 h in a block heater (Gehrke et al., 1985) with high-purity 6 M HCl (5 mL) after
189 flushing with N₂ gas. For Met and Cys, aliquots containing 2 mg N and the internal standard
190 were preoxidized with 1 mL performic acid (0.9 mL of 88% formic acid, 0.1 mL of 30% H₂O₂
191 and 5 mg phenol) for 16 h at 4 °C prior to acid hydrolysis as described above (Mason et al.,
192 1980, Elkin and Griffith, 1984). After hydrolysis, tube contents were filtered through Whatman
193 541 filter paper and filtrate was diluted to 50 mL in a volumetric flask with HPLC grade H₂O.
194 Aliquots (0.5 mL) were evaporated at 60 °C under constant N₂ flushing, with 3 rinses and re-
195 evaporations with HPLC grade H₂O to remove acid residues. After final evaporation, hydrolysate
196 was dissolved in 1 mL of Na diluent (Na220, Pickering Laboratories, Mountain View, CA).

197 Individual AA hydrolysates were separated using an Agilent 1100 series HPLC (Agilent
198 Technologies, Santa Clara, CA) fitted with a sodium cation exchange column (Cat. no
199 1154110T, Pickering Laboratories, Mountain View, CA) using a 4 buffer step gradient and

200 column temperature gradient. Detection of separated AA was performed at 560 nm following
201 post-column ninhydrin derivation. Standards (250 nM/mL) for the individual amino acids were
202 prepared by diluting a pure standard in sample buffer. The volume of sample and standards
203 loaded onto the column was 10 μ L. For Trp determination, a separate aliquot of sample
204 containing 2 mg N was hydrolyzed with 1.2 g of Ba(OH)₂ at 110 °C for 16 h on a block heater
205 according to the method of Landry and Delhaye (1992). Included in the hydrolysis was 125 μ L
206 of 5-Methyl-Trp (5 mM) as an internal standard. After cooling to precipitate barium ions, an
207 aliquot (3 μ L) of the hydrolysate was added to 1 mL of acetate buffer (0.07 M sodium acetate)
208 and analyzed using fluorescence detection (excitation = 285 nm, emission = 345 nm) after HPLC
209 separation.

210 *Calculations*

211 Calculation of ¹⁵N atom percent excess (**APE**) in rumen contents, omasal fractions and
212 microbial samples; omasal nutrient flow, and partitioning of NAN are described in the
213 companion paper (Fessenden et al., 20XXa). Briefly, total N entering the omasal canal was
214 partitioned into three fractions: ammonia N, microbial N, and non-ammonia non-microbial N
215 (**NANMN**). Total NAN flow was calculated as the difference between total N and ammonia N.

216 The N content of bacteria and protozoa was used to calculate flow of OM and DM in each
217 microbial fraction. Partitioning of the microbial pool into bacterial and protozoal pools takes into
218 account the differences in ¹⁵N APE between bacteria and protozoa samples, therefore reducing
219 the underestimation bias introduced by assuming bacterial ¹⁵N enrichment as representative of all
220 microbial biomass (Brito et al., 2007). Protozoal predation was estimated using the ¹⁵N
221 enrichment of the microbial fractions in the following manner

$$\text{Engulfed bacterial N} = \frac{\text{protozoa N flow (g/d)} \times \text{protozoa }^{15}\text{N APE (g/g)} \times 0.9 / 0.5}{\text{bacteria }^{15}\text{N APE (g/g)}}$$

223 In the preceding calculation, it is assumed that 90% of the enriched ¹⁵N in the protozoa is of
 224 bacterial origin; recognizing the capability of protozoa for de-novo synthesis of AA from
 225 ammonia (Williams and Harfoot, 1976; Williams and Coleman, 1997; Newbold et al., 2005).
 226 The calculation also assumes that 50% of the engulfed N is incorporated into cell N (Hristov and
 227 Jouany, 2005), an assumption also incorporated into a dynamic version of the CNCPS (Higgs,
 228 2014). Protozoa consumption of bacterial DM and OM was determined using the N and OM
 229 content of the omasal bacteria.

230 Rumen OM, fermentable carbohydrate (**CHO**), NAN, and microbial NAN pool sizes were
 231 determined from nutrient analysis of the samples taken during the rumen evacuations. Measured
 232 ¹⁵N APE of the total rumen NAN pool was used to partition microbial and non-microbial N in
 233 the same manner as described for omasal NAN flows. Pool size calculations for digestible OM
 234 and CHO are as follows:

$$\text{Digestible OM (kg)} = \text{Rumen OM (kg)} - \text{Microbial OM pool (kg)} - \text{rumen uNDFom pool (kg)}$$

$$\text{Digestible CHO (kg)} = \text{Rumen digestible OM pool (kg)} - (\text{Rumen CP pool} - \text{Microbial CP pool}) - (\text{rumen DM pool} * \text{diet fat content (g/g of DM)})$$

239 To estimate the partition of the rumen microbial N pool into bacteria and protozoal pools,
 240 relative flows of bacteria and protozoa were multiplied by a factor representing selective
 241 retention of protozoa in the rumen. Reported rumen protozoa N retention in rumen vs. post-
 242 ruminal measurements vary widely, and range from < 5 % (Sylvester et al., 2005) to over 70%
 243 (Punia et al., 1992). Therefore, rumen protozoa ¹⁵N proportion of the total rumen ¹⁵N pool (**PP**)

244 was calculated at 4 different levels, from no selective retention to 75 % selective retention. To
 245 make this estimation, the ratio of protozoa ¹⁵N flow to total omasal ¹⁵N flow was divided by 1,
 246 0.75, 0.5, and 0.25, representing selective retention of 0, 25, 50, and 75%, respectively:

247 Protozoa ¹⁵N proportion of the total rumen ¹⁵N, (PP; g/g) = [Protozoa ¹⁵N flow (g/d) / OTD
 248 ¹⁵N flow (g/d)] / (1,0.75,0.5,0.25)

249 The protozoa proportion of the rumen ¹⁵N at each of the 4 levels of selective retention, along
 250 with the APE of rumen contents and the microbial fractions were then used to calculate the
 251 rumen pool sizes for bacteria, protozoa, and total microbial NAN:

252 Protozoa NAN pool size (g) = [Rumen contents ¹⁵N APE (g/g) x Rumen total N (g) x PP,
 253 (g/g)] / protozoa ¹⁵N APE (g/g)

254 Bacteria NAN pool size (g) = [Rumen contents ¹⁵N APE (g/g) x Rumen total N (g) x (1-PP,
 255 g/g)] / bacteria ¹⁵N APE (g/g)

256 Microbial NAN pool size (g) = Protozoa NAN pool size (g) + Bacteria NAN pool size (g)

257 The value obtained when using a selective retention rate of 25% was used in calculations
 258 requiring a total rumen microbial pool size. Justification for this approach is discussed later in
 259 this paper. Rumen pool size and omasal flow was then used to calculate the fractional growth
 260 rate of total microbial, bacteria and protozoa fractions:

261 Fractional growth rate (h⁻¹) =
$$\frac{\text{flow of microbial, bacterial, or protozoal N, g/h}}{\text{Rumen pool size of microbial, bacterial, or protozoal N g}}$$

262 Since flows and pool sizes were measured values, the fractional growth rate accounts for
 263 lyses and turnover in the rumen. The same pool size and flow approach was used to calculate the
 264 absolute and fractional degradation rates of OM and CHO in the rumen, where the numerator

265 was the hourly rate of disappearance of OM or CHO, and the denominator is the rumen pool size
266 of digestible OM or CHO. Fractional rate of CHO degradation and fractional rate of microbial
267 growth was then used to calculate yield of microbial cells per gram of CHO degraded (Y_g)

$$268 \quad Y_g \text{ (g cell DM / g CHO degraded)} = \frac{\text{fractional rate of microbial growth}}{\text{fractional rate of CHO degradation}}$$

269 Flows of individual AA in OTD was calculated using the concentration of AA in each
270 omasal fraction and the triple marker system described in Fessenden et al (20XXa). Bacteria,
271 protozoa, total microbial, and non-microbial AA flow were then calculated as follows:

$$272 \quad \text{Bacterial AA flow (g/d)} = \text{bacteria N flow (g/d)} \times \text{bacteria AA (g/g N)}$$

$$273 \quad \text{Protozoa AA flow (g/d)} = \text{protozoa N flow (g/d)} \times \text{protozoa AA (g/g N)}$$

$$274 \quad \text{Microbial AA flow (g/d)} = \text{protozoa AA flow (g/d)} + \text{bacteria AA flow (g/d)}$$

$$275 \quad \text{Non-microbial AA flow (g/d)} = \text{OTD AA flow (g/d)} - \text{microbial AA flow (g/d)}$$

276 At the end of the experiment, all relevant farm, cattle, and diet information were entered into
277 an experimental version of the CNCPS v. 7 (Higgs, 2014) to provide comparisons with reported
278 values. The model mechanistically describes substrate degradation using rates of passage and
279 degradation (Waldo et al., 1972), and relates microbial growth to substrate availability (Russell
280 et al., 1992) with modifications (Higgs, 2014). Protozoa, endogenous N transactions, N
281 recycling, and large intestine degradation of substrate are all represented in a mechanistic
282 manner. To provide the model with rates of CHO degradation, feedstuffs were analyzed using
283 methods available from commercial laboratories. The fermentation time points used to calculate
284 the rates of digestion for aNDFom were 30, 120 and 240 h (Raffrenato et al., 2018). Starch
285 disappearance after 7 h of in vitro rumen incubation was used to calculate a fractional rate of

286 degradation (Fessenden 2011; Sniffen and Ward 2011). Comparisons were made of model
287 predicted vs. measured values for substrate degradation, microbial growth, and post-ruminal AA
288 flows.

289 *Statistical Analyses*

290 All data were analyzed using SAS version 9.3 (SAS Institute Inc. Cary, NC). The same
291 model as described in Fessenden et al. (20XXa) is reproduced here:

$$292 Y_{ijkl} = \mu + S_i + C_{j:i} + P_k + T_l + ST_{il} + \varepsilon_{ijkl}$$

293 where Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of sequence i , $C_{j:i}$ = random
294 effect of cow within sequence, P_k = fixed effect of period k , T_l = fixed effect of treatment l , ST_{il}
295 = fixed interaction effect of sequence i and treatment l , and ε_{ijkl} = residual error. Degrees of
296 freedom were calculated using the Kenward-Roger option. Means were determined using the
297 least squares means statement, and treatment means were compared using the PDIFF option.
298 Statistical significance was considered at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

299 **RESULTS AND DISCUSSION**

300 *Microbial Nutrient Composition*

301 The OM content of omasal bacteria and protozoa did not differ between diets, and averaged
302 84.1 and 87.6%, respectively (Table 2). Organic matter content was similar to values obtained
303 previously from rumen and omasal isolates (Brito et al., 2006; 2007), although OM content is
304 strongly influenced by the isolation procedures used (Martin et al., 1994). Nitrogen content (% of
305 DM) of bacteria and protozoa were both affected by diet, with increased N content in microbial
306 isolates from cows fed EXP. When expressed on an OM basis, protozoa N did not differ between
307 cows fed EXP or CON. This increased N content in bacteria could arise from several possible

308 mechanisms. With decreased protein degradation in cows fed EXP as reported in Fessenden et al.
309 (20XXa), it is possible that less protein was degraded completely to ammonia, thus increasing
310 AA and peptides available for microbial incorporation, leading to increased N content. Brito et
311 al. (2007) reported a similar 5% increase in NAN content of fluid associated bacteria when urea
312 vs. true protein supplements were fed to lactating dairy cows. An alternative possibility is a
313 change in microbial reserve carbohydrate synthesis, resulting in more glycogen to dilute the
314 measured NAN value. In microbial competition studies for substrate, Denton et al. (2015)
315 demonstrated that protozoa sequestered up to 60% of available glucose and stored it as glycogen,
316 while less than 2% was recovered in bacteria. In this study, cows fed CON demonstrated lower
317 microbial N content, which could indicate more reserve CHO synthesis. This could also provide
318 a possible explanation for the relative change in bacteria vs. protozoa NAN content when
319 expressed on an OM basis. Bacteria N (% OM) increased by 3.5% (9.45 vs. 9.79% of OM for
320 cows fed CON vs. EXP, respectively), while protozoa NAN content did not differ between diets.
321 Differential amounts of glycogen synthesis by bacteria vs. protozoa could lead to the observed
322 result. Omasal protozoa NAN content has been reported as low as 2.3% of DM (Brito et al.,
323 2006), although sucrose was used in the isolation procedure, likely influencing N content. The
324 protozoa isolation method employed in the current study deliberately omitted any addition of
325 glycogenic compounds to avoid biasing the N measurement. Further examination into the rate
326 and extent of uptake of AA and glucogenic precursors by microbial communities might be
327 warranted. Enrichment of ^{15}N in bacteria and protozoa was similar for cows fed CON vs. EXP.
328 The mean protozoal:bacterial ^{15}N enrichment ratio of 0.62 was within the range of values
329 reported in the literature: 0.40 (Ahvenjärvi et al., 2002), 0.63 (Hristov and Broderick, 1996), 0.75
330 (Cecava et al., 1991). Some authors attributed low enrichment to feed contamination in the

331 isolation method, although ^{15}N enrichment is likely more related to the sources of N used for
332 growth (Atasoglu et al., 2001; Brito et al., 2006) and possibly the amount of time the bacteria
333 had to take up the label. The approach used in this experiment followed the concept of a plateau
334 in enrichment and cows were infused for 72 h before any measurements were made (Marini and
335 Van Amburgh, 2003; Recktenwald et al. 2014), thus different enrichment levels were likely not
336 due to non-plateau of ^{15}N . Further, Recktenwald (2010) demonstrated that the rumen $\text{NH}_3\text{-N}$
337 pool reaches equilibrium rapidly after bolus dosing with doubly-labeled urea. In this study, it is
338 unlikely that relatively small changes in the ammonia pool, as reported in the companion paper,
339 would lead to bias in enrichment measurements due to ^{15}N dilution.

340 Total AA content in bacteria and protozoa was increased in cows fed EXP vs. CON. Amino
341 acid content as a percent of N was not different between diets, and averaged 50.3 and 54.0% for
342 omasal bacteria and protozoa, respectively. These values are lower than reported previously in
343 the literature (Storm and Ørskov, 1983). Hvelplund (1986) reported a mean AA N as a percent of
344 N of 67.4% in mixed rumen bacteria, and demonstrated a curvilinear relationship of diet starch
345 and sugar content vs. AA N as a percent of N, with AA N decreasing rapidly as starch+sugar
346 content exceeded 30% of diet DM. Reporting of AA N in this experiment did not include DAPA,
347 which can represent greater than 10% of the amino acid content in rumen micro-organisms.
348 Another explanation for the lower AA N content of the microbes could be related to the
349 procedure used in this experiment. Formalin has been previously shown to affect the AA
350 composition of isolated cells (Stern et al., 1983), likely through cross-linkage of protein chains.
351 Volden and Harstad (1998) reported an 11% decrease in total AA N as a percent of N when
352 formalin treatment was used in the isolation procedure, with some individual AA such as Lys,
353 Tyr, decreasing more than 30%. Additionally, the formalin treatment can create products

354 resistant even to acid hydrolysis, rendering incomplete extraction of AA from the sample matrix
355 (Barry, 1976; Fessenden et al., 2017). Therefore, bacterial and protozoal AA profiles and
356 contributions to AA flow might be underestimated in this study; however total flow of AA in
357 OTD is unaffected, as no formalin was used in the separation of LQ, SP, or LP fractions.
358 Overall, this data on AA N composition of microbes suggests the use of formalin in the isolation
359 procedure should be eliminated if AA analysis and composition is an objective of the
360 experiment.

361 Omasal bacteria and protozoa AA composition was unaffected by diet (Table 3). Profiles of
362 bacterial amino acids generally agree well with literature reports (Volden et al., 1999) with the
363 exception of Lys and Tyr, which were decreased in the current study and formalin is known to
364 specifically affect these AA (Volden and Harstad, 1998). The protozoal AA profile was similar
365 when compared with bacterial AA profile, with the exception of Met and Lys showing
366 numerically increased levels in protozoa. Volden et al. (1999) also reported increased Lys
367 concentration in protozoa vs. bacteria; however Met was very similar between isolates in that
368 study. Cockburn and Williams (1984) reported mean Met concentrations of 2.4 g / 100 g AA
369 which is very consistent with the average value of the two treatments in this study, 2.45 g / 100 g
370 AA. Inconsistent use and poor reporting of pre-oxidation procedures used among studies makes
371 many comparisons of sulfur AA difficult, as recoveries from pre-oxidation are rarely reported
372 (Spindler et al., 1984). It is possible that formalin treatment increased the relative proportion of
373 Met in our microbial isolates; however this effect should also be distributed across other AA not
374 affected by formalin treatment.

375 *Omasal Flows of AA in OTD, Microbial, and Non-microbial Fractions*

376 Microbial nutrient flow of DM, OM and NAN flow was not different between diets (Table
377 4). Effects of rumen degradable protein source on omasal nutrient flows have been previously
378 described in Fessenden et al (20XXa). Cows fed CON vs. EXP tended to have increased
379 bacterial DM and OM flow (4808 vs. 4056 g DM/d and 4023 vs. 3433 g OM/d for CON vs.
380 EXP, respectively). Since bacterial OM and DM flow are calculated using microbial N as a
381 marker, it is likely that the observed difference in microbial N composition contributed to lower
382 calculated DM and OM flows. Protozoa nutrient flows followed similar numeric trends, but
383 flows did not differ significantly between diets. Protozoa accounted for 15.8 and 17.9% of the
384 total microbial NAN flow in cows fed CON vs. EXP, respectively. This estimate is slightly
385 higher than has previously been reported in the literature with animals at similar levels of intake.
386 Sylvester et al. (2005) reported protozoa N accounting for 5.9 to 11.9% of the microbial N flow
387 using 18S rDNA techniques, while Ahvenjärvi et al. (2002) reported 7% of microbial N flow as
388 protozoa N using a very similar technique as our study; albeit with much different diets. Very
389 few studies attempt to directly measure protozoa flows due to the difficulty of the approach. As
390 such, some researchers have taken alternative approaches to estimate protozoal contribution to
391 the microbial N pool. Using a linear programming approach, Shabi et al. (2000) estimated
392 protozoal N to account for 7 to 19% of microbial N flow. This was a result similar to that
393 estimated by Steinhour et al. (1982) using a differential ¹⁵N enrichment approach, although many
394 assumptions were made pertaining to pool size and turnover in that study. Alternatively,
395 computer simulations by Dijkstra et al. (1998) indicated that protozoa N could account for 10.7
396 to 26.1% of microbial N in cattle at 17.1 kg of DMI. Simulations using CNCPS v. 7 indicated
397 that overall microbial flow in cows fed CON was well predicted; however the model was
398 insensitive to the numerical difference in microbial flow in CON vs. EXP fed cows (Table 4).

399 Protozoa flow (g/d) was underpredicted by 43%. It is possible that the coefficients used to
400 calculate protozoa growth are relatively low as they are often based on in vitro studies of the
401 more easily cultured protozoa species (Coleman and Hall, 1984; Coleman, 1992). The rates of
402 substrate uptake and growth reported in vitro experiments might be considerably lower than
403 those achieved rumen. It is also possible that the predictions of protozoa passage from the rumen
404 are not correct in CNCPS v7 because there are few data on which to build robust equations and
405 the model structure uses particle passage as a basis whereas, protozoa might be passing in the
406 liquid phase, which would lead to underestimations in the current predictions. Future studies
407 measuring microbial N flows should report protozoal contribution to the microbial flow, as data
408 in this area are lacking.

409 The flow of AA in OTD is presented in Table 5. Most AA demonstrated increased flow in
410 cows fed EXP vs. CON. Total AA flow was increased by 211 g/d in cows fed EXP compared to
411 CON (2456 v. 2245 g/d for CON vs. EXP, respectively; $P < 0.01$). Omasal flow of Lys, Met, and
412 Phe, were similar between diets, while all other EAA were increased in cows fed EXP compared
413 with CON. Total non-essential AA flow was increased by 116 g/d in cows fed EXP, while Cys
414 flow was the only individual NEAA that was similar between diets. Reynal and Broderick (2005)
415 reported similar results in omasal AA flows when diets with varying RDP from soybean meal vs.
416 treated soybean meal were fed to lactating dairy cows. When soybean meal, cottonseed meal,
417 and canola meal were compared with a urea control, flows of all AA increased greatly in a study
418 by Brito et al. (2007). The increase in AA flows in the current study was directly related to the
419 lower dietary CP degradation in cows fed EXP, as reported by Fessenden et al. (20XXa). It is
420 notable that while total NAN flow was not different between diets, total AA flow was increased
421 in cows fed fermentation byproduct. This would suggest that the NAN flow in cows fed the CON

422 diets might have a higher content of non-AA nitrogenous compounds. The observed results
423 might also occur if AA with higher N content are preferentially degraded in the rumen of cows
424 fed fermentation byproduct, thus reducing the measured NAN flow. Overall, fermentation
425 byproduct inclusion increased non-microbial AA flow relative to control. (Table 6). Inclusion of
426 the fermentation byproduct had no effect on microbial AA flow, while non-microbial AA flow
427 was increased for most individual AA. This further supports the protein sparing effect of the
428 fermentation byproduct on RDP, as microbial AA flow was not significantly lower, while non-
429 microbial AA flow increased by 316 g/d in cows fed EXP compared to CON ($P = 0.03$).
430 Microbial protein synthesis was apparently not negatively affected by decreased CP degradation,
431 indicating that sufficient AA and N compounds were present to support high rates of microbial
432 growth. It should be noted that the non-microbial AA flows presented in Table 6 are calculated
433 by subtraction of microbial AA flow from total AA flow. As such, experimental and
434 measurement errors will be disproportionally represented in the reported non-microbial AA
435 flows. This might also lead to values for individual AA that are outside biologically expected
436 ranges. This occurred with Trp, where the flow (determined by difference of total AA flow vs.
437 microbial AA flow) would result in a negative number.

438 *Omasal Flows of AA in Bacteria and Protozoa*

439 Flows of individual amino acids in bacterial and protozoa fractions were generally not
440 affected by diet (Table 7). Bacterial Leu flow was increased in cows fed CON vs. EXP (63.4 vs.
441 45.1 g/d for CON vs. EXP, respectively; $P = 0.03$). Bacterial Ser and Tyr flows tended to be
442 increased in cows fed CON vs. EXP, while all other bacterial AA flows were not different
443 between diets. Protozoa AA flows were unaffected by treatment. The contribution of protozoa to
444 total microbial AA flow is in Table 8. Inclusion of the fermentation byproduct in the EXP diets

445 increased protozoa contribution to Leu and Lys flow. Protozoa flow of Lys accounted for 21.5
446 vs. 29.5% of the total microbial flow of Lys in cows fed EXP vs. CON, respectively, ($P < 0.01$).
447 Contribution of protozoa to total EAA flow tended to be greater in cows fed EXP vs. CON (19.0
448 vs. 22.8% of microbial flow, respectively; $P = 0.07$). These results demonstrate the importance
449 of protozoa to post ruminal AA flows. Protozoa NAN contribution to microbial NAN flows
450 averaged 16.9% of microbial N, while contribution of protozoa AA to microbial AA flows
451 ranged from 8 to 46% for individual AA. Models that do not take into account the difference in
452 AA profile, composition, and contribution of protozoa to microbial AA flow might have poor
453 predictions of post-ruminal AA flow. For models seeking to describe the diet adequacy to
454 support milk protein production, accurate predictions of post ruminal supply are needed if the
455 models are to be applied in practical feeding situations (Pacheco et al., 2012). Higgs (2014)
456 evaluated CNCPS v. 7 against a large literature dataset, and found the model adequately predicts
457 post ruminal total NAN and microbial NAN supply; however individual AA were generally
458 over-predicted. The same tendency to over-predict AA flow was observed in the current
459 evaluation might be indicative of mean bias of the model, or could be related to a methodological
460 bias associated with the use of the triple-marker system, as all studies in the dataset utilized this
461 approach.

462 ***Rumen Pool Size and Kinetics of OM, CHO, Bacteria and Protozoa***

463 Rumen pool sizes of digestible OM, CHO, NAN, and microbial NAN were not affected by
464 diet (Table 9). Generally, microbial N pool size of 3.5 g of microbial N/L was similar to that
465 observed by Sylvester et al. (2005), who reported a microbial pool of approximately 3.0 g of
466 microbial N/L, although the authors point out that the estimation of bacterial N contains the error
467 of both the 18S RNA procedure and the purine determination. Purines have been shown to be

468 inconsistent microbial markers relative to ^{15}N (Klopfenstein et al., 2001; Firkins and Reynolds,
469 2005; Ipharraguerre et al., 2007). Cows fed CON had greater microbial DM contribution to the
470 total DM pool than cows fed EXP (27.7 vs. 23.6 % for CON vs. EXP, respectively; $P = 0.05$).
471 The values in this experiment were within the range of 17 to 27 % of the rumen DM pool as
472 microbial DM reported by Craig et al. (1987).

473 Selective retention of protozoa in the rumen is not well understood, and estimates range from
474 < 5 % (Sylvester et al., 2005) to over 70% (Punia et al., 1992). Since rumen protozoa mass was
475 not quantified directly in this study, the effect of 4 different levels of retention is described in
476 Table 9. Further, since total rumen microbial pool size was estimated from ^{15}N enrichment of the
477 rumen NAN pool, it is possible to evaluate the effect of selective retention on pool sizes,
478 assuming total microbial ^{15}N pool size remains constant. Therefore, at 0% selective retention, we
479 expect the protozoa to account for the same proportion of total rumen microbial N as measured
480 in OTD flow, while at greater levels of retention, protozoa account for larger portions of the
481 microbial pool. Bacteria pool size was decreased in cows fed EXP vs. CON, and bacteria vs.
482 protozoa pool sizes diverged as estimated selective retention increased. At the highest estimation
483 of selective retention, protozoa were calculated to represent 55 to 58% of the total microbial pool
484 (CON vs. EXP, respectively, $P = 0.40$).

485 To assess which level of selective retention of protozoa is likely most correct, it was possible
486 to use pool size and flow to estimate fractional rates of turnover (Table 10). In this case, since
487 actual flows and microbial pool size were measured, the rate of turnover of the pool can be used
488 as an index of microbial growth rate (Wells and Russell, 1996). Recognizing that the main
489 energy substrate for rumen bacteria is CHO (Russell et al., 1992), and assuming the maximum
490 yield of cell DM per g of CHO degraded is 0.5 (Isaacson et al., 1975), one can quickly determine

491 which retention values allows for realistic growth rates. In this instance, selective retention at
492 50% indicate that bacteria would have to grow at a fractional rate of 0.07 h^{-1} , corresponding to a
493 CHO degradation rate of 0.14 h^{-1} ($0.07 / 0.5$). Given the estimated pool size (g) and digestion
494 (g/h), the fractional rate of CHO availability in this study averaged 0.138 h^{-1} of the available
495 pool; therefore theoretical maximal fractional growth rate was estimated at 0.138×0.5 , or ~
496 0.069 h^{-1} . Using the measured total microbial pool at 25% selective retention, it was calculated
497 that the fractional growth rate of all microbes in the rumen was 0.061 h^{-1} . This corresponds to an
498 estimated Y_g of 0.44 g/g of CHO degraded. This value is close to the theoretical maximums for
499 individual species reported in pure cultures (Russell and Baldwin, 1979; Theodorou and France,
500 2005). In vitro measurements of mixed rumen microbes often give yields on the high range of
501 those observed in pure culture (Russell and Wallace, 1997). The range of yields (29 to 100 g /
502 mol of hexose) reported by Russell and Wallace (1997), correspond to Y_g of 0.16 to 0.55 g / g of
503 glucose degraded. Stouthamer (1973) estimated a maximal Y_g of approximately 0.8 g / g of
504 glucose using biochemical pathways, indicating the possibility for much higher yields in some
505 bacterial species; however values this high are rarely reported in vitro with mixed rumen
506 microbial fermentations. It is possible that higher growth rates could be achieved in vivo, as it
507 can be very difficult to maintain ideal conditions for microbial growth outside the rumen.

508 The CNCPS relates cell growth directly to CHO availability in the manner described above,
509 so accurate estimates of CHO degradation are key to accurately predicting microbial yield.
510 Simulations indicated that the model characterized CHO digestion reasonably well (Table 10)
511 with predicted absolute rates of degradation approximately 37 grams lower than observed. This
512 corresponds to a fractional rate of degradation of CHO at 0.124 h^{-1} . Given the predicted
513 microbial yield (Table 4), the apparent Y_g used by the model was 0.45 g / g of CHO degraded in

514 the rumen; very similar to the value observed in vivo. Unfortunately, many other in vivo studies
515 investigating rumen outflows do not report rumen pool sizes or individual microbial populations,
516 thereby limiting the utility of the data for model evaluation. Even so, the limited data presented
517 here shows good agreement of predicted vs. independent measured values, indicating that the
518 structure of the model is likely adequate to provide accurate estimates of microbial yield from
519 substrate degradation. This provides a strong basis from which to improve AA supply
520 predictions, as microbial AA represents a large portion of metabolizable AA flowing from the
521 rumen.

522 CONCLUSIONS

523 The inclusion of the fermentation byproduct in EXP diets increased microbial N and AA N
524 flows compared with CON cows fed a urea control. Microbial AA composition did not differ
525 between diets; however estimates of total AA N and specific AA were likely lower than
526 literature values due to formalin treatment. Daily flows of AA were increased in OTD as a result
527 of decreased degradation of feed N as reported by Fessenden et al. (20XXa). This was reflected
528 in the current study with increased non-microbial AA flow in cows fed EXP vs. CON. Average
529 protozoa contribution to microbial NAN flow was 16.8%, yet protozoa AA accounted for 21% of
530 the microbial EAA flow, with a range of 8 to 46% for individual AA. Cows in this study
531 maintained an average pool size of 320 g of microbial N in the rumen, while bacterial and
532 protozoal pools were estimated at 4 different theoretical levels of retention. Fractional growth
533 rate of all microbes in the rumen was measured at 0.069 h^{-1} , with a Yg of 0.44 g / g of CHO
534 degraded. A dynamic versions of the CNCPS v. 7 was able to accurately predict CHO
535 degradation and total microbial yield, however improvements are needed for bacteria vs.
536 protozoa partitioning and individual AA flow predictions. Overall, the current structure of the

537 CNCPS v. 7 provides a strong base for predicting supply of microbial NAN. Future model
538 improvements in microbial AA profiles, intestinal digestibility, protozoa partitioning, and dietary
539 protein degradation might be needed to improve estimates of individual AA flow.

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REFERENCES

Ahvenjärvi, S., A. Vanhatalo, and P. Huhtanen. 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. *J. Anim. Sci.* 80:2176-2187. <http://dx.doi.org/10.2527/2002.8082176x>

AOAC International. 2005. Official methods of analysis. 18th ed. AOAC International, Gaithersburg, MD.

Atasoglu, C., C. J. Newbold, and R. J. Wallace. 2001. Incorporation of [15N] ammonia by the cellulolytic ruminal bacteria *Fibrobacter succinogenes* BL2, *Ruminococcus albus* SY3, and *Ruminococcus flavefaciens* 17. *Appl. Environ. Microbiol.* 67:2819-2822. <http://dx.doi.org/10.1128/AEM.67.6.2819-2822.2001>.

Barry, T. 1976. The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. *Proc Nutr Soc.* 35:221-229. <http://dx.doi.org/10.1079/PNS19760035>.

Brito, A. F., G. A. Broderick, and S. M. Reynal. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on omasal flow and microbial protein synthesis in dairy cows. *J. Dairy Sci.* 89:3939-3953. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72436-5](http://dx.doi.org/10.3168/jds.S0022-0302(06)72436-5).

Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007. Effects of different protein supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1828-1841. <http://dx.doi.org/10.3168/jds.2006-559>.

Broderick, G. A., N. De Leon, and Y. Nakamura. 2000. Potential of fermentation byproducts as nitrogen supplements for lactating dairy cows. *J. Dairy Sci.* 83:2548-2556. [http://dx.doi.org/10.3168/jds.S0022-0302\(00\)75147-2](http://dx.doi.org/10.3168/jds.S0022-0302(00)75147-2).

568 Cecava, M., N. Merchen, L. Berger, R. Mackie, and G. Fahey. 1991. Effects of dietary energy
569 level and protein source on nutrient digestion and ruminal nitrogen metabolism in steers.
570 *J. Anim. Sci.* 69:2230-2243. <http://dx.doi.org/10.2527/1991.6952230x>.

571 Cockburn, J. E. and A. P. Williams. 1984. The simultaneous estimation of the amounts of
572 protozoal, bacterial and dietary nitrogen entering the duodenum of steers. *Br. J. Nutr.*
573 51:111-132. <http://dx.doi.org/10.1079/BJN19840014>.

574 Cotta, M. A. and J. B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen
575 bacterial protein synthesis in continuous culture. *J. Dairy Sci.* 65:226-234.
576 [http://dx.doi.org/10.3168/jds.S0022-0302\(82\)82181-4](http://dx.doi.org/10.3168/jds.S0022-0302(82)82181-4).

577 Coleman, G. S. 1992. The rate of uptake and metabolism of starch grains and cellulose particles
578 by entodinium species, eudiplodinium maggii, some other entodiniomorphid protozoa
579 and natural protozoal populations taken from the ovine rumen. *J. Appl. Microbiol.*
580 73:507-513. <https://doi.org/10.1111/j.1365-2672.1992.tb05013.x>.

581 Coleman, G. S. and F. J. Hall. 1984. The uptake and utilization of entodinium caudatum,
582 bacteria, free amino acids and glucose by the rumen ciliate entodinium bursa. *J. Appl.*
583 *Microbiol.* 56:283-294. <https://doi.org/10.1111/j.1365-2672.1984.tb01349.x>.

584 Craig, W. M., G. A. Broderick, and D. B. Ricker. 1987. Quantitation of microorganisms
585 associated with the particulate phase of ruminal ingesta. *J. Nutr.* 117:56-62.

586 Denton, B. L., L. E. Diese, J. L. Firkins, and T. J. Hackmann. 2015. Accumulation of reserve
587 carbohydrate by rumen protozoa and bacteria in competition for glucose. *Appl. Environ.*
588 *Microbiol.* 81:1832-1838. <http://dx.doi.org/10.1128/AEM.03736-14>.

589 Dijkstra, J., J. France, and S. Tamminga. 1998. Quantification of the recycling of microbial
590 nitrogen in the rumen using a mechanistic model of rumen fermentation processes. *J.*
591 *Agric. Sci.* 130:81-94.

592 Elkin, R. and J. Griffith. 1984. Hydrolysate preparation for analysis of amino acids in sorghum
593 grains: effect of oxidative pretreatment. *J. AOAC* 68:1117-1121.

594 Fessenden, S. W. 2011. Effects of corn prolamin content on in vitro starch digestibility as
595 measured by various methods. B.S. Honors Thesis, Cornell Univ., NY.

596 Fessenden, S. W. 2016. Amino acid supply in dairy cattle. Ph.D. Dissertation. Cornell Univ.,
597 Ithaca, NY. <https://ecommons.cornell.edu/handle/1813/45365>.

598 Fessenden, S. W. and M. E. Van Amburgh. 2016. Characterization of non-nutritive factors of
599 feeds for model development. Pages 155-169 in *Proc. Cornell Nutrition Conference*,
600 Syracuse, NY. Cornell University, Ithaca, NY.
601 <https://ecommons.cornell.edu/handle/1813/44741>.

602 Fessenden S.W., T. J. Hackmann, D. A. Ross , A. Foskolos , M. E. Van Amburgh. 2017.
603 Ruminant bacteria and protozoa composition, digestibility, and amino acid profile
604 determined by multiple hydrolysis times. *J Dairy Sci.* 100:7211-7226. doi:
605 10.3168/jds.2016-12531.

606 Firkins, J. L. and C. K. Reynolds. 2005. Whole animal nitrogen balance in cattle. Pages 167–185
607 in *Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of*
608 *Cattle Operations*. E. Pfeffer and A. N. Hristov, ed. CAB International, Wallingford, UK.

609 France, J. and R. Siddons. 1986. Determination of digesta flow by continuous marker infusion. *J.*
610 *Theor. Biol.* 121:105-119. [http://dx.doi.org/10.1016/S0022-5193\(86\)80031-5](http://dx.doi.org/10.1016/S0022-5193(86)80031-5).

611 Gehrke, C. W., L. Wall Sr, J. Absheer, F. Kaiser, and R. Zumwalt. 1985. Sample preparation for
612 chromatography of amino acids: acid hydrolysis of proteins. *J. AOAC* 68:811-821.

613 Higgs, R. 2014. Development of a dynamic rumen and gastro-intestinal model in the Cornell Net
614 Carbohydrate and Protein System to predict the nutrient supply and requirements of dairy
615 cattle. Ph.D. Dissertation. Cornell Univ., Ithaca, NY.

616 Higgs, R., L. Chase, D. Ross, and M. Van Amburgh. 2015. Updating the Cornell Net
617 Carbohydrate and Protein System feed library and analyzing model sensitivity to feed
618 inputs. *J. Dairy Sci.* 98:6340-6360. <http://dx.doi.org/10.3168/jds.2015-9379>.

619 Hristov, A. N. and G. A. Broderick. 1996. Synthesis of microbial protein in ruminally cannulated
620 cows fed alfalfa silage, alfalfa hay, or corn silage. *J. Dairy Sci.* 79:1627-1637.
621 [http://dx.doi.org/10.3168/jds.S0022-0302\(96\)76526-8](http://dx.doi.org/10.3168/jds.S0022-0302(96)76526-8).

622 Hristov, A. N. and J.-P. Jouany. 2005. Nitrogen requirements of cattle. Pages 117-166 in *Factors*
623 *Affecting the Efficiency of Nitrogen Utilization in the Rumen*. A. Pfeffer and A. N.
624 Hristov, ed. CABI, Wallingford, UK.

625 Huhtanen, P., P. G. Brotz, and L. D. Satter. 1997. Omasal sampling technique for assessing
626 fermentative digestion in the forestomach of dairy cows. *J. Anim. Sci.* 75:1380-1392.
627 <http://dx.doi.org/10.2527/1997.7551380x>.

628 Hvelplund, T. 1986. The influence of diet on nitrogen and amino acid content of mixed rumen
629 bacteria. *Acta Agric. Scand.* 36:325-331. <http://dx.doi.org/10.1080/00015128609436535>.

630 Ipharraguerre, I. R., S. M. Reynal, M. Lineiro, G. A. Broderick, and J. H. Clark. 2007. A
631 comparison of sampling sites, digesta and microbial markers, and microbial references
632 for assessing the postruminal supply of nutrients in dairy cows. *J. Dairy Sci.* 90:1904-
633 1919. <http://dx.doi.org/10.3168/jds.2006-159>.

634 Isaacson, H. R., F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy
635 utilization by mixed rumen bacteria in continuous culture. *J. Dairy Sci.* 58:1645-1659.
636 [http://dx.doi.org/10.3168/jds.S0022-0302\(75\)84763-1](http://dx.doi.org/10.3168/jds.S0022-0302(75)84763-1).

637 Klopfenstein, T. J., R. A. Mass, K. W. Creighton, and H. H. Patterson. 2001. Estimating forage
638 protein degradation in the rumen. *J. Anim. Sci.* 79:E208-E216.
639 <http://dx.doi.org/10.2527/jas2001.79E-SupplE208x>.

640 Landry, J. and S. Delhaye. 1992. Simplified procedure for the determination of tryptophan of
641 foods and feedstuffs from barytic hydrolysis. *J. Agric. Food Chem.* 40:776-779.
642 <http://dx.doi.org/10.1021/jf00017a014>.

643 Lean, I. J., T. K. Webster, W. Hoover, W. Chalupa, C. J. Sniffen, E. Evans, E. Block, and A. R.
644 Rabiee. 2005. Effects of BioChlor and Fermenten on microbial protein synthesis in
645 continuous culture fermenters. *J. Dairy Sci.* 88:2524-2536.
646 [http://dx.doi.org/10.3168/jds.S0022-0302\(05\)72930-1](http://dx.doi.org/10.3168/jds.S0022-0302(05)72930-1).

647 Lobley, G., D. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as
648 assessed by refined, non-invasive [¹⁵N¹⁵N] urea kinetics. *Br. J. Nutr.* 84:459-468.
649 <http://dx.doi.org/10.1017/S0007114500001768>.

650 Marini, J. C. and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein
651 heifers. *J Anim. Sci.* 81:545-552. <http://doi:10.2527/2003.812545x>.

652 Martin, C., A. G. Williams, and B. Michalet-Doreau. 1994. Isolation and characteristics of the
653 protozoal and bacterial fractions from bovine ruminal contents. *J. Anim. Sci.* 72:2962-
654 2968. <http://dx.doi.org/10.2527/1994.72112962x>.

655 Mason, V. C., S. Bech-Andersen, and M. Rudemo. 1980. Hydrolysate preparation for amino acid
656 determinations in feed constituents. *Z Tierphysiol Tierernahr Futtermittelkd.* 43:146-164.
657 <http://dx.doi.org/10.1111/j.1439-0396.1980.tb00618.x>.

658 Newbold, C. J., N. R. McEwan, R. E. Calza, E. N. Chareyron, S. M. Duval, S. C. Eschenlauer, F.
659 M. McIntosh, N. Nelson, A. J. Travis, and R. J. Wallace. 2005. An NAD⁺-dependent
660 glutamate dehydrogenase cloned from the ruminal ciliate protozoan, *Entodinium*
661 *caudatum*. *FEMS Microbiol. Lett.* 247:113-121.
662 <http://dx.doi.org/10.1016/j.femsle.2005.04.034>.

663 Pacheco, D., R. A. Patton, C. Parys, and H. Lapierre. 2012. Ability of commercially available
664 dairy ration programs to predict duodenal flows of protein and essential amino acids in
665 dairy cows. *J. Dairy Sci.* 95:937-963. <http://dx.doi.org/10.3168/jds.2011-4171>.

666 Penner, G., L. Guan, and M. Oba. 2009. Effects of feeding Fermenten on ruminal fermentation in
667 lactating Holstein cows fed two dietary sugar concentrations. *J. Dairy Sci.* 92:1725-1733.
668 <http://dx.doi.org/10.3168/jds.2008-1706>.

669 Punia, B. S., J. Leibholz, and G. J. Faichney. 1992. Rate of production of protozoa in the rumen
670 and the flow of protozoal nitrogen to the duodenum in sheep and cattle given a pelleted
671 diet of lucerne hay and barley. *J Agric Sci.* 118:229-236.

672 Raffrenato, E., D. A. Ross, and M. E. Van Amburgh. 2018. Development of an in vitro method
673 to determine rumen undigested aNDFom for use in feed evaluation. *J. Dairy Sci.*
674 101:9888-9000. <https://doi.org/10.3168/jds.2018-15101>.

675 Reynal, S. M. and G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on
676 production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 88:4045-4064.
677 [http://dx.doi.org/10.3168/jds.S0022-0302\(05\)73090-3](http://dx.doi.org/10.3168/jds.S0022-0302(05)73090-3).

678 Recktenwald, E. B. 2010. Urea N recycling and its utilization by ruminal microbial populations
679 in lactating dairy cattle. Ph.D. Dissertation, Cornell Univ., Ithaca, NY.

680 Recktenwald, E. B., D. A. Ross, S. W. Fessenden, C. J. Wall, and M. E. Van Amburgh. 2014.
681 Urea N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude
682 protein and starch with or without monensin. *J. Dairy Sci.* 97:1611-1622.
683 <https://doi.org/10.3168/jds.2013-7162>.

684 Russell, J. and R. Wallace. 1997. Energy-yielding and energy-consuming reactions. Pages 246–
685 282 in *The Rumen Microbial Ecosystem*. 2nd ed. P. N. Hobson and C. S. Stewart, ed.
686 Blackie Academic & Professional, New York, NY.

687 Russell, J. B. and R. L. Baldwin. 1979. Comparison of maintenance energy expenditures and
688 growth yields among several rumen bacteria grown on continuous culture. *Appl. Environ.*
689 *Microbiol.* 37:537-543.

690 Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net
691 carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J.*
692 *Anim. Sci.* 70:3551-3561. <http://dx.doi.org/10.2527/1992.70113551x>.

693 Shabi, Z., H. Tagari, M. R. Murphy, I. Bruckental, S. J. Mabjeesh, S. Zamwel, K. Celik, and A.
694 Arieli. 2000. Partitioning of amino acids flowing to the abomasum into feed, bacterial,
695 protozoal, and endogenous fractions. *J. Dairy Sci.* 83:2326-2334.
696 [http://dx.doi.org/10.3168/jds.S0022-0302\(00\)75120-4](http://dx.doi.org/10.3168/jds.S0022-0302(00)75120-4).

697 Siddons, R. C., J. Paradine, D. E. Beever, and P. R. Cornell. 1985. Ytterbium acetate as a
698 particulate-phase digesta-flow marker. *Br. J. Nutr.* 54:509-519.
699 <http://dx.doi.org/10.1079/BJN19850136>.

700 Sniffen, C. J., and R. Ward. 2011. Using starch digestibility information in ration balancing.
701 Pages 121-133 in Proc. Western Canadian Dairy Seminar: Advances in Dairy
702 Technology, Red Deer, AB.

703 Spindler, M., R. Stadler, and H. Tanner. 1984. Amino acid analysis of feedstuffs: Determination
704 of methionine and cystine after oxidation with performic acid and hydrolysis. *J. Agric.*
705 *Food Chem.* 32:1366-1371. <http://dx.doi.org/10.1021/jf00126a038>.

706 Steinhour, W. D., M. Stokes, J. Clark, J. Rogers, C. Davis, and D. Nelson. 1982. Estimation of
707 the proportion of non-ammonia-nitrogen reaching the lower gut of the ruminant derived
708 from bacterial and protozoal nitrogen. *Br. J. Nutr.* 48:417-431.
709 <http://dx.doi.org/10.1079/BJN19820124>.

710 Stern, M. D., L. M. Rode, R. W. Prange, R. H. Stauffacher, and L. D. Satter. 1983. Ruminant
711 protein degradation of corn gluten meal in lactating dairy cattle fitted with duodenal t-
712 type cannulae. *J. Anim. Sci.* 56:194-205.

713 Storm, E. and E. R. Ørskov. 1983. The nutritive value of rumen micro-organisms in ruminants 1.
714 Large-scale isolation and chemical composition of rumen micro-organisms. *Br. J. Nutr.*
715 50:463-470. <http://dx.doi.org/10.1079/BJN19830114>.

716 Stouthamer, A.H., 1973. A theoretical study on the amount of ATP required for synthesis of
717 microbial cell material. *Antonie Van Leeuwenhoek.* 39:545-565.
718 <http://dx.doi.org/10.1007/BF02578899>.

719 Sylvester, J. T., S. K. R. Karnati, Z. Yu, C. J. Newbold, and J. L. Firkins. 2005. Evaluation of a
720 real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal
721 nitrogen. *J. Dairy Sci.* 88:2083-2095. [http://dx.doi.org/10.3168/jds.S0022-](http://dx.doi.org/10.3168/jds.S0022-0302(05)72885-X)
722 [0302\(05\)72885-X](http://dx.doi.org/10.3168/jds.S0022-0302(05)72885-X).

723 Theodorou, M. and J. France. 2005. Rumen microorganisms and their interactions. Pages 145–
724 163 in *Quantitative Aspects of Ruminant Digestion and Metabolism* J. M. Forbes and J.
725 France, ed. CAB International, Wallingford, UK.

726 Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R.
727 Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein
728 System: A model for precision feeding of dairy cattle. *Anim. Feed Sci. Technol.* 143:174-
729 202. <http://dx.doi.org/10.1016/j.anifeedsci.2007.05.010>.

730 Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt
731 as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625-632.
732 <http://dx.doi.org/10.1002/jsfa.2740310702>.

733 Van Amburgh, M., E. Collao-Saenz, R. Higgs, D. Ross, E. Recktenwald, E. Raffrenato, L.
734 Chase, T. Overton, J. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and
735 Protein System: Updates to the model and evaluation of version 6.5. *J. Dairy Sci.*
736 98:6361-6380. <http://dx.doi.org/10.3168/jds.2015-9378>.

737 VandeHaar, M. J. and N. St-Pierre. 2006. Major advances in nutrition: Relevance to the
738 sustainability of the dairy industry. *J. Dairy Sci.* 89:1280-1291.
739 [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72196-8](http://dx.doi.org/10.3168/jds.S0022-0302(06)72196-8).

740 Volden, H. and O. M. Harstad. 1998. Amino acid composition of bacteria harvested from the
741 rumen of dairy cows fed three diets differing in protein content and rumen protein
742 degradability at two levels of intake. *Acta Agric. Scand. A Anim. Sci.* 48:210-215.
743 <http://dx.doi.org/10.1080/09064709809362422>.

744 Volden, H., O. M. Harstad, and L. T. Mydland. 1999. Amino acid content and profile of
745 protozoal and bacterial fractions isolated from ruminal contents of lactating dairy cows

746 fed diets differing in nitrogen supplementation. *Acta Agric. Scand. A Anim. Sci.* 49:245-
747 250. <http://dx.doi.org/10.1080/090647099424006>.

748 Waldo, D. R., L. W. Smith, and E. L. Cox. 1972. Model of cellulose disappearance from the
749 rumen. *J. Dairy Sci.* 55:125-129. [http://dx.doi.org/10.3168/jds.S0022-0302\(72\)85442-0](http://dx.doi.org/10.3168/jds.S0022-0302(72)85442-0).

750 Wells, J. E. and J. B. Russell. 1996. Why do many ruminal bacteria die and lyse so quickly? *J.*
751 *Dairy Sci.* 79:1487-1495. [http://dx.doi.org/10.3168/jds.S0022-0302\(96\)76508-6](http://dx.doi.org/10.3168/jds.S0022-0302(96)76508-6).

752 Whitehouse, N., V. Olson, C. Schwab, W. Chesbrot, K. Cunningham, and T. Lykos. 1994.
753 Improved techniques for dissociating particle-associated mixed ruminal microorganisms
754 from ruminal digesta solids. *J. Anim. Sci* 72:1335-1343.
755 <http://dx.doi.org/10.2527/1994.7251335x>.

756 Williams, A. G. and G. S. Coleman. 1997. The rumen protozoa. Pages 73-139 in *The Rumen*
757 *Microbial Ecosystem*. P. N. Hobson and C. S. Stewart, ed. Elsevier Science Publishers
758 Ltd., London, UK. <http://dx.doi.org/>

759 Williams, A. G. and C. G. Harfoot. 1976. Factors affecting the uptake and metabolism of soluble
760 carbohydrates by the rumen ciliate *Dasytricha ruminantium* isolated from ovine rumen
761 contents by filtration. *Microbiology* 96:125-136. <http://dx.doi.org/10.1099/00221287-96->
762 1-125.

763

Table 1. Ingredient and nutrient composition (mean \pm SD)¹ of experimental diets

Item	Diet	
	CON	EXP
Ingredient, % DM		
Corn silage	44.6	44.6
Alfalfa silage	12.0	12.0
Corn meal	12.0	12.0
Expeller soybean meal ²	8.0	8.0
Soybean hulls	5.8	5.8
Citrus pulp, dry	3.3	3.3
Chocolate meal	2.4	2.4
Saturated fatty acid ³	1.2	1.2
Molasses	0.9	0.9
Blood meal	1.7	1.7
Wheat middlings	4.8	3.2
Fermentation byproduct ⁴	–	3.0
Calcium carbonate	–	0.7
Urea	0.4	–
Calcium sulfate, dihydrate	1.7	–
Sodium bicarbonate	0.33	0.40
Salt white	0.30	0.32
Magnesium oxide	0.17	0.17
Dicalcium phosphate	0.16	0.16
Supplemental methionine ⁵	0.06	0.06
Vitamin and mineral mix ⁶	0.18	0.18
Nutrient composition		
DM, %	44.5 \pm 0.7	44.2 \pm 0.8
OM, % of DM	93.9 \pm 0.3	93.8 \pm 0.6
CP, % of DM	15.9 \pm 0.6	16.1 \pm 0.5
RDP, % of DM ⁷	8.4 \pm 0.1	8.0 \pm 0.1
Starch, % of DM	27.5 \pm 1.1	27.8 \pm 0.5
Sugars, % of DM	5.4 \pm 0.4	5.3 \pm 0.3
NFC, % of DM ⁷	41.7 \pm 0.2	41.8 \pm 1.3
aNDFom, % of DM	30.9 \pm 0.2	31.2 \pm 0.2
ADF, % of DM	19.9 \pm 1.5	19.7 \pm 0.6
ADL, % of NDF	10.0 \pm 0.9	10.0 \pm 1.4
Ether extract, % of DM	5.0 \pm 0.2	4.9 \pm 0.2
ME, Mcal/kg ⁷	2.5 \pm 0.1	2.5 \pm 0.1

¹Analyzed values from 3 period composite samples. Table is from Fessenden et al. (20XXa)

²SOYPLUS (West Central Cooperative, Ralston, IA).

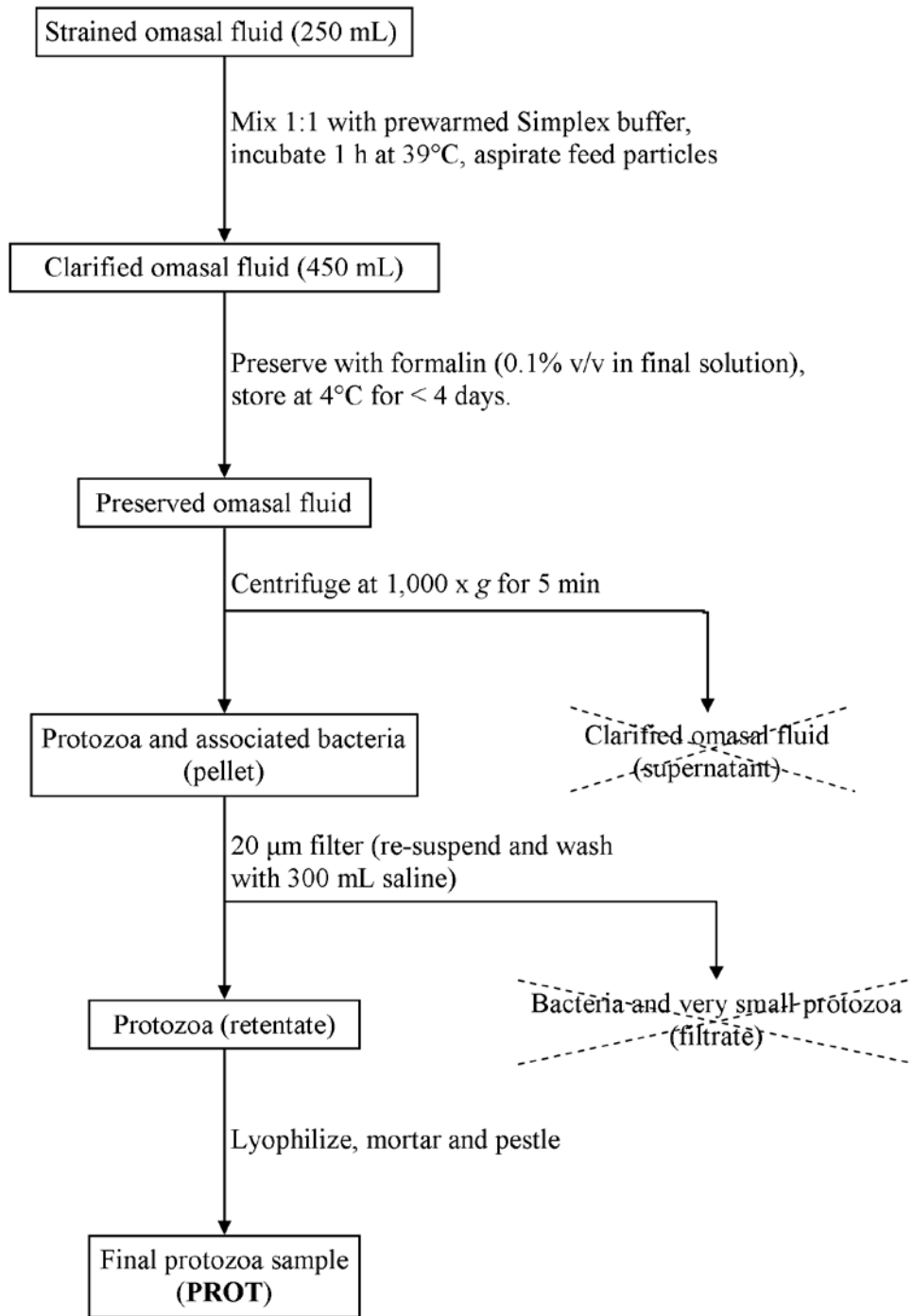
³ENERGY BOOSTER 100 (MSC Company, Dundee, IL).

⁴FERMENTEN (Church & Dwight, Inc., Princeton, NJ).

⁵SMARTAMINE M (Bluestar Adisseo Nutrition Group, Alpharetta, GA).

⁶Provided (per kg of diet DM): 44 mg of Zn, 32 mg of Mn, 10 mg of Cu, 1 mg of Co, 1 mg of I, 0.3 mg of Se, 5000 IU of vitamin A, 980 IU of vitamin B, and 25 IU of vitamin E.

⁷Calculated by the Cornell Net Carbohydrate and Protein System v. 6.5.



764

765 **Figure 3.1. Flowchart for preparation of protozoa isolates.** Fractions discarded are crossed

766 out.

Table 2. Chemical composition and isotopic enrichment of omasal bacteria and protozoa in lactating dairy cattle fed two different sources of rumen available nitrogen

Item	Diet ¹		SEM	P
	CON	EXP		
Bacteria				
OM, % of DM	83.6	84.7	0.5	0.18
N, % of DM	7.90	8.29	0.13	0.02
N, % of OM	9.45	9.79	0.14	0.04
¹⁵ N atom% excess	0.035	0.034	0.002	0.74
Total AA, % of DM	28.8	30.8	0.4	0.01
Total AA, % of OM	34.3	36.3	0.4	0.02
AA, % of N	50.1	50.6	0.8	0.67
Protozoa				
OM, % of DM	86.1	89.1	1.5	0.17
N, % of DM	7.93	8.60	0.24	0.05
N, % of OM	9.21	9.65	0.24	0.24
¹⁵ N atom% excess	0.023	0.020	0.002	0.11
Total AA, % of DM	31.4	34.4	0.9	0.02
Total AA, % of OM	36.4	38.6	0.9	0.12
AA, % of N	53.4	54.6	0.9	0.32

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Table 3. Omasal bacteria and protozoa amino acid composition in lactating dairy cattle fed two different sources of rumen available nitrogen

Item	Bacteria AA, g/100 g AA				Protozoa AA, g/100 g AA			
	Diet ¹		SEM	<i>P</i>	Diet ¹		SEM	<i>P</i>
	CON	EXP			CON	EXP		
Essential AA								
ARG	5.60	5.38	0.23	0.49	5.16	5.84	0.36	0.20
HIS	1.96	2.02	0.04	0.30	2.67	2.67	0.12	0.96
ILE	4.70	4.66	0.06	0.60	5.15	4.91	0.25	0.51
LEU	4.42	3.60	0.30	0.07	5.59	5.61	0.40	0.96
LYS	4.13	4.04	0.05	0.24	6.15	6.16	0.26	0.98
MET	2.34	2.56	0.16	0.34	3.99	4.00	0.10	0.93
PHE	6.50	6.44	0.15	0.79	7.08	6.85	0.14	0.29
TRP	4.94	4.98	0.13	0.85	3.74	3.63	0.09	0.26
THR	6.08	5.93	0.21	0.62	4.79	4.90	0.25	0.75
VAL	6.55	6.71	0.09	0.16	5.83	6.05	0.22	0.49
Total EAA	47.2	46.3	0.6	0.26	50.1	50.8	0.4	0.23
Nonessential AA								
ALA	7.07	7.18	0.08	0.36	5.66	5.62	0.17	0.86
ASP	10.65	10.67	0.32	0.95	11.45	11.87	0.67	0.62
CYS	1.20	1.14	0.06	0.49	2.84	2.80	0.05	0.62
GLU	14.36	14.59	0.15	0.30	13.87	14.11	0.28	0.49
GLY	5.77	5.89	0.05	0.10	4.84	4.84	0.13	0.99
PRO	7.22	8.57	0.46	0.04	3.96	2.73	0.95	0.37
SER	5.31	4.81	0.20	0.10	4.49	4.74	0.20	0.40
TYR	1.15	0.82	0.16	0.02	2.72	2.60	0.20	0.67
Total NEAA	52.8	53.7	0.60	0.26	49.9	49.2	0.40	0.23

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Table 4. Omasal microbial nutrient flows and protozoa predation in lactating dairy cattle fed two different sources of rumen available nitrogen

Item	Diet ¹		SEM	<i>P</i>
	CON	EXP		
Dry matter intake, kg/d ²	23.8	23.9	0.7	0.91
Total microbial nutrient flow, g/d				
DM flow	5,718	4,930	358	0.14
OM flow	4,815	4,210	310	0.19
NAN flow ²	450	409	28	0.31
Bacterial nutrient flow, g/d				
DM flow	4,808	4,056	286	0.08
OM flow	4,023	3,433	240	0.10
NAN flow	378	337	23.0	0.22
% of microbial N flow	84.2	82.1	1.0	0.12
Protozoa nutrient flow, g/d				
DM flow	909	850	82	0.61
OM flow	790	764	81	0.82
NAN flow	72.1	73.9	7.3	0.84
% of microbial N flow	15.8	17.9	1.0	0.12
Protozoa predation of bacteria, g/d				
DM consumed	1,159	929	166	0.33
OM consumed	967	783	138	0.35
N consumed	90.6	76.3	12.9	0.45
% of bacterial N flow	23.4	22.2	2.4	0.70
CNCPS v. 7 output				
Predicted microbial N flow, g/d	412	417	-	-
Bacteria N flow	371	375	-	-
Protozoa N flow	41	42	-	-
% of microbial N flow	9.9	10.1	-	-
Predation estimate, bacterial N consumed, g/d	75	76	-	-

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

²Previously reported in Fessenden et al. (20XXa)

Table 5. Effect of rumen available nitrogen source on omasal true digesta flow of AA in lactating dairy cattle

AA flow, g/d	Diet ¹		SEM	<i>P</i>
	CON	EXP		
Essential AA				
ARG	108.2	120.1	3.4	0.01
HIS	62.2	68.0	1.5	< 0.01
ILE	87.3	100.2	2.7	< 0.01
LEU	131.0	149.2	7.0	0.03
LYS	139.0	127.4	14.0	0.56
MET	57.3	57.3	5.5	1.00
PHE	147.7	165.8	8.3	0.11
TRP	78.9	86.3	2.4	< 0.01
THR	119.9	131.6	3.3	< 0.01
VAL	116.8	131.1	3.6	< 0.01
Total EAA	1,045.8	1,141.8	28.4	0.03
Nonessential AA				
ALA	140.9	153.0	4.0	< 0.01
ASP	212.3	236.2	5.7	< 0.01
CYS	39.3	38.0	3.4	0.78
GLU	280.1	314.0	6.3	< 0.01
GLY	105.9	116.5	2.7	< 0.01
PRO	172.1	189.4	6.2	0.03
SER	120.0	131.4	3.3	< 0.01
TYR	130.4	138.7	4.4	0.05
Total NEAA	1,200.9	1,316.8	31.4	< 0.01
Total AA	2,245.2	2,456.5	57.1	< 0.01

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Table 6. Effect of rumen available nitrogen source on omasal flow of microbial and non-microbial AA in lactating dairy cattle

Item	Microbial AA flow, g/d				Non-microbial AA flow, g/d			
	Diet ¹		SEM	<i>P</i>	Diet ¹		SEM	<i>P</i>
	CON	EXP			CON	EXP		
Essential AA								
ARG	96.9	88.4	6.7	0.38	10.7	31.8	7.9	0.08
HIS	36.4	34.7	2.3	0.57	25.8	33.2	2.7	0.04
ILE	83.1	75.4	5.7	0.34	4.2	23.4	6.4	0.01
LEU	79.5	61.7	6.3	0.06	52.9	87.9	9.7	0.02
LYS	78.4	71.9	5.8	0.42	60.5	56.3	14.7	0.84
MET	43.1	44.8	3.4	0.67	14.0	12.8	6.1	0.88
PHE	114.0	103.0	7.8	0.35	33.4	61.9	10.4	0.06
TRP	82.7	74.5	5.2	0.28	–	11.4	6.7	–
THR	103.3	92.9	7.8	0.35	16.4	37.0	8.9	0.05
VAL	112.4	106.3	8.0	0.58	4.3	23.1	10.0	0.09
Total EAA	830.2	749.0	51.4	0.28	213.6	371.7	64.6	0.04
Nonessential AA								
ALA	119.8	111.2	8.0	0.43	20.5	40.5	9.7	0.07
ASP	188.7	177.8	13.8	0.51	23.1	56.7	15.5	0.04
CYS	24.8	23.0	1.5	0.34	14.4	15.3	3.7	0.86
GLU	249.8	234.9	17.7	0.51	30.0	76.7	19.9	0.04
GLY	98.1	91.7	6.8	0.48	7.6	23.7	7.8	0.08
PRO	117.0	122.1	9.6	0.66	55.7	65.8	12.7	0.42
SER	90.5	79.5	6.7	0.17	29.5	50.7	8.3	< 0.01
TYR	24.2	19.4	2.5	0.09	106.2	119.1	4.4	< 0.01
Total NEAA	913.1	861.0	61.7	0.49	287.1	445.6	73.7	0.03
Total AA	1,744.2	1,611.4	113.9	0.39	500.0	815.9	135.4	0.03

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Table 7. Effect of rumen available nitrogen source on omasal flow of bacteria and protozoa AA flow in lactating dairy cattle

Item	Bacteria AA flow, g/d				Protozoa AA flow, g/d			
	Diet ¹		SEM	<i>P</i>	Diet ¹		SEM	<i>P</i>
	CON	EXP			CON	EXP		
Essential AA								
ARG	81.8	70.5	5.9	0.20	15.3	18.4	2.5	0.35
HIS	28.6	26.5	2.0	0.45	7.8	8.0	0.6	0.77
ILE	68.0	60.2	4.7	0.27	15.0	15.1	1.6	0.95
LEU	63.4	45.1	5.4	0.03	16.1	16.6	1.4	0.79
LYS	60.2	53.4	4.3	0.26	18.1	18.6	1.9	0.85
MET	31.5	32.5	2.8	0.77	11.7	12.1	1.2	0.84
PHE	93.3	82.5	6.4	0.25	20.6	20.7	1.9	0.96
TRP	71.8	63.5	4.7	0.23	10.9	11.0	1.0	0.91
THR	89.0	77.4	6.4	0.21	14.2	15.1	1.9	0.71
VAL	95.1	87.2	6.5	0.41	17.1	18.6	2.2	0.60
Total EAA	682.9	597.1	42.2	0.17	146.9	154.1	14.8	0.71
Nonessential AA								
ALA	103.1	93.7	6.9	0.34	16.6	17.1	1.9	0.86
ASP	154.8	141.7	10.6	0.31	34.0	36.2	4.6	0.69
CYS	16.6	14.4	1.1	0.17	8.3	8.4	0.7	0.93
GLU	208.8	191.5	14.9	0.38	40.9	42.9	4.3	0.70
GLY	83.8	76.4	5.7	0.38	14.3	14.9	1.7	0.77
PRO	105.5	114.0	10.3	0.50	11.4	8.2	2.4	0.37
SER	77.2	64.5	5.7	0.10	13.3	14.3	1.7	0.66
TYR	16.1	11.2	2.5	0.06	8.1	8.0	1.0	0.94
Total NEAA	766.1	708.6	51.5	0.39	147.1	149.5	15.0	0.90
Total AA	1,449.7	1,304.3	93.6	0.27	293.9	303.7	29.8	0.80

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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Table 8. Effect of rumen available nitrogen source on protozoa proportion of omasal microbial AA flow in lactating dairy cattle

Protozoa AA contribution to microbial AA flow, %	Diet ¹		SEM	P
	CON	EXP		
Essential AA				
ARG	17.0	22.0	2.0	0.07
HIS	22.5	25.9	1.7	0.18
ILE	18.8	21.5	1.6	0.15
LEU	21.5	29.5	1.6	< 0.01
LYS	23.9	28.2	1.6	0.05
MET	28.6	31.3	2.5	0.46
PHE	19.0	22.0	1.7	0.22
TRP	17.8	24.4	4.1	0.27
THR	14.7	17.8	1.5	0.15
VAL	16.0	19.0	1.4	0.17
Total EAA	19.0	22.8	1.5	0.07
Nonessential AA				
ALA	14.7	17.1	1.4	0.25
ASP	18.9	22.6	1.8	0.09
CYS	34.4	40.0	2.4	0.11
GLU	17.2	20.1	1.5	0.15
GLY	15.2	17.8	1.5	0.23
PRO	10.6	7.7	2.7	0.46
SER	15.6	21.0	1.7	0.04
TYR	35.4	45.8	5.5	0.21
Total NEAA	16.8	19.4	1.3	0.20
Total AA	17.8	20.9	1.4	0.13

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Table 9. Effect of rumen available nitrogen source on rumen pool sizes of organic matter, carbohydrate, and non-ammonia nitrogen in lactating dairy cattle

Item	Diet ¹		SEM	P
	CON	EXP		
Rumen pool sizes				
Digestible OM, kg ²	6.61	7.06	0.50	0.50
Total fermentable CHO, kg ³	3.98	4.10	0.32	0.79
Total NAN, g	586	614	38	0.53
Microbial NAN pool at 25% selective retention, g	340	300	21	0.21
Microbial DM proportion of rumen DM pool, %	27.7	23.6	1.4	0.05
Bacteria NAN rumen pool sizes, g ⁴				
0% selective retention	281	240	18	0.13
25% selective retention	271	229	17	0.11
50% selective retention	250	209	17	0.07
75% selective retention	181	148	17	0.07
Protozoa NAN rumen pool sizes, g ⁵				
0% selective retention	53	53	5	0.98
25% selective retention	70	70	7	0.98
50% selective retention	105	105	10	0.98
75% selective retention	210	211	21	0.98
Protozoa NAN pool, % of total microbial NAN pool				
0% selective retention	15.8	17.9	1.0	0.12
25% selective retention	20.7	23.2	1.3	0.13
50% selective retention	30.1	33.1	1.8	0.17
75% selective retention	55.1	58.0	2.9	0.40

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

²Measured OM from rumen evacuation, corrected for microbial OM and uNDF240

³Rumen OM pool – (Rumen CP pool – Microbial CP pool) – (rumen DM pool * diet fat content)

⁴Microbial NAN pool – Protozoa NAN pool at 4 levels of selective retention of protozoa

⁵Microbial NAN x Protozoa % of omasal flow x level of selective retention

Table 10. Fractional rates of microbial growth, nutrient digestion, and rumen fermentation parameters in lactating dairy cattle fed two different sources of rumen available nitrogen

Item	Diet ¹		SEM	P
	CON	EXP		
Fractional growth rate of bacteria ² , h ⁻¹				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.064	0.064	0.005	0.99
50% selective retention	0.070	0.070	0.006	1.00
75% selective retention	0.108	0.103	0.012	0.74
Fractional growth rate of protozoa ² , h ⁻¹				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.046	0.046	0.003	0.99
50% selective retention	0.030	0.030	0.002	0.99
75% selective retention	0.015	0.015	0.001	0.99
Omasal flows and ruminal digestion parameters				
True OM flow, kg/d ³	7.08	7.19	0.47	0.87
Microbial NAN flow, g/d ³	450	409	28	0.31
Ruminal true OM digestion rate, g/h	626	619	17	0.77
Ruminal true CHO digestion rate, g/h	518	526	15	0.72
Fractional rate of OM digestion ⁴ , h ⁻¹	0.101	0.094	0.008	0.54
Fractional rate of CHO digestion ⁴ , h ⁻¹	0.139	0.138	0.011	0.91
Microbial growth parameters				
Fractional growth rate, h ⁻¹	0.060	0.060	0.004	0.94
Theoretical maximum CHO allowable growth ⁵ , h ⁻¹	0.070	0.069	0.005	0.91
Observed Yg, g of cells / g of CHO degraded ⁶	0.44	0.44	0.03	0.99
% of theoretical maximum Yg	88.4	88.3	6.6	0.99
CNCPS v. 7 output				
Predicted CHO degradation, g/h	484	487	-	-
Predicted fractional rate of CHO digestion, h ⁻¹	0.124	0.124	-	-
Predicted Yg, g of cells / g of CHO degraded	0.45	0.45	-	-

¹CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

²bacteria or protozoa daily flow (g/h) / bacteria or protozoa pool size (g) at 4 levels of protozoa selective retention

³Previously reported in Fessenden et al. (20XXa)

⁴Measured microbial NAN flow (g/h) / measured rumen microbial NAN pool (g)

⁵Fractional rate of CHO digestion x 0.5

⁶Fractional microbial growth rate / fractional rate of CHO digestion