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A meta-analysis of variability in continuous-culture ruminal fermentation and digestibility data

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ABSTRACT

A meta-analysis was conducted to compare ruminal fermentation and digestibility data and variability between continuous-culture (CC) experiments and in vivo data. One hundred eighty CC studies representing 1,074 individual treatments, published in refereed journals between 1980 and 2010 were used in this analysis. Studies were classified into 2 groups based on the type of CC used: CC systems specified as rumen simulation techniques (RUSITEC) and non-RUSITEC CC systems (non-RUSITEC). The latter was a diverse group of systems, all of which were termed CC by the investigators. The CC data were compared with a data set of in vivo trials with ruminally cannulated lactating dairy cows (data from a total of 366 individual cows). The reported neutral detergent fiber (NDF) concentration of the diets fed in the 3 data sets was, on average (dry matter basis), 44, 34, and 32%, respectively. The average total volatile fatty acid (VFA) concentration for the RUSITEC and non-RUSITEC data sets was 67 and 80% (respectively) of the total VFA concentration in vivo. The average concentration of acetate was also lower for the CC data sets compared with in vivo and that of propionate was considerably lower for RUSITEC compared with in vivo, but butyrate concentrations were similar between the CC and in vivo data sets. Variability in the VFA data was generally the highest (higher coefficients of variation and variance) for the non-RUSITEC data set, followed by RUSITEC, and was the lowest for in vivo. Digestibilities of NDF and particularly organic matter were lower in the CC data sets compared with in vivo; the average NDF digestibility was 34.2, 45.5, and 53.0% for RUSITEC, non-RUSITEC, and in vivo, respectively. Variability in nutrient digestibility data followed the pattern of variability of the VFA data: highest variability for the non-RUSITEC data set, followed by RUSITEC, and the lowest for in vivo. This analysis showed that CC systems are generally characterized by lower total VFA and acetate concentrations, extremely low counts or lack of ruminal protozoa, and lower organic matter and NDF digestibilities than in vivo. Overall, variability was much greater for CC than for in vivo experimental data.

Key words: continuous culture, ruminal fermentation, digestibility, meta-analysis

INTRODUCTION

Continuous-culture (**CC**) systems are a subcategory of rumen simulation techniques fitting the definition given by R. E. Hungate (i.e., ". . . a vat in which fresh feed and saliva mix with the fermenting mass, and fluid and feed residues leave in quantities equivalent to those entering"; Hungate, 1966). The main advantages of these techniques are (1) the ability to test a large number of treatments, in sufficient replication, and in a short period of time; (2) the ability to test higher, in some cases potentially toxic to the animal, levels of a given feed additive; and (3) low experimental cost (compared with an animal trial). A major advantage of a CC system, compared with a batch-culture in vitro system, is the ability to remove fermentation end products and maintain a relatively stable fermentation for prolonged periods (Czerkawski and Breckenridge, 1977). Due to various factors inherent in all in vitro systems, however, the original microbial community may degenerate in a CC system and protozoa disappear (Slyter and Putnam, 1967; Mansfield et al., 1995), although some designs are able to better maintain microbial (including protozoal) diversity (Teather and Sauer, 1988; Muetzel et al., 2009). Continuous-culture systems of various designs have been widely used to evaluate the effects of diet, feed composition, and feed supplements on ruminal digestion, microbial protein synthesis, and ruminal fermentation (Benchaar et al., 2009). As an example, a search of the Commonwealth Agricultural Bureau International database (CABI; http://www.

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cabi.org/default.aspx?site=170&page=999, animal sciences subject area; accessed Jan. 27, 2012) with (1) "continuous culture" and "rumen" and (2) "RUSITEC" and "rumen" as keyword combinations, returned a total of 533 hits. Many important differences exist between CC and in vivo [e.g., lack of absorption, differences in fluid and particle dilution (passage) rates, and feed intake per rumen volume] that can influence the digestive processes. For example, the amount of substrate per unit of fermentation medium volume is very small in CC compared with in vivo rumen simulation techniques (**RUSITEC**; 20 g of substrate/d per 0.7 L = 29 g/L; average dairy cow, 20,000 g per 80 L = 250 g/L). To apply CC data to in vivo conditions, it is important to know how well CC results correspond to in vivo data. In many instances, feed supplement use is recommended based on CC data, although results may not correspond to in vivo data. Several studies have published simultaneously CC and in vivo data (although the goal may not have been to directly compare the 2 systems) and will be used as examples here to illustrate the above point. Devant et al. (2001) investigated protein sources (soybean meal vs. fish and corn gluten meals) in CC compared with in vivo in beef cattle and reported significantly different concentrations of acetate, propionate, and acetate:propionate ratio in CC, but lack of effect of protein source on rumen VFA in vivo. Similarly, the effects of protein source and urea supplementation (also a treatment) on the estimated microbial protein synthesis in the rumen were highly significant in the CC experiment, but no difference was observed in vivo. Another study reported increased fiber digestibility due to malic acid supplementation in CC, but no effect was observed in vivo (Sniffen et al., 2006). Other examples of discrepancies between CC and in vivo data in the same study are available in the literature (microbial protein synthesis: Dann et al., 2006; Molina-Alcaide et al., 2009; ruminal fermentation and digestibility: Carro et al., 2009; Cantalapiedra-Hijar et al., 2011). To our knowledge, however, a systematic analysis of CC fermentation and digestibility data has not been conducted. Thus, the objective of this meta-analysis was to compare ruminal fermentation and digestibility data and variability between CC studies and data from in vivo experiments with lactating dairy cows.

MATERIALS AND METHODS

One hundred and eighty CC studies representing 1,074 individual treatments, published in refereed journals between 1980 and 2010, were used in this analysis. All studies used CC methods to investigate effects of diet or feed additives on ruminal fermentation, microbial populations, and nutrient digestibility. Studies were classified into 2 groups based on the type of CC used: CC system specified as RUSITEC (Czerkawski and Breckenridge, 1977) and non-RUSITEC CC systems. The latter was a diverse group of systems, all of which were termed CC by the investigators. The main difference of the RUSITEC system, compared with other CC systems, is primarily in the method of solid feed supplementation (i.e., incubation in nylon bags). The design of the non-RUSITEC systems used in the analysis was not studied. For comparative purposes, a data set of in vivo trials with ruminally-cannulated lactating dairy cows was constructed. The in vivo data set included a total of 366 individual cow observations for the ruminal fermentation data and a maximum of 352 observations for total-tract apparent digestibility. The in vivo trials were conducted at the University of Idaho (Moscow), The Pennsylvania State University (University Park), and The Ohio State University (Columbus) (Hristov et al., 2001b, 2004a,b, 2005, 2008, 2009, 2010, 2011a,b,c; Hristov and Ropp, 2003; Foley et al., 2006; Vander Pol et al., 2008; Oelker et al., 2009; Agle et al., 2010a,b; Lee et al., 2011; Mathew et al., 2011; Tekippe et al., 2011; Reveneau et al., 2012a,b). Cows in this data set consumed, on average, 23 kg of DM/d (SD = 5) and milked 34 kg/d (SD = 10) with 3.3% milk fat (SD = 0.6) and 3.0% milk true protein (SD = 0.3). Additionally, digestibility data from the CC data sets were compared with published in vivo total-tract apparent digestibility data from a meta-analysis of European trials with lactating dairy cows (a maximum of 497 treatment means; Huhtanen et al., 2009).

Data were analyzed and removed from the data sets as outliers based on an absolute studentized residual value >2 (PROC REG procedure of SAS; SAS Institute Inc., Cary, NC). For the remaining data, PROC MEANS was used for descriptive statistical analysis (mean, SD, and CV) and PROC MIXED was used to analyze variability, or variance component, within each data set. The model included the measured response, type of data set (RUSITEC, non-RUSITEC, or in vivo), the residual error (assumed to be distributed normally with a mean of 0 and constant variance σ^2), REPEATED statement with the GROUP option (type of data set), and RANDOM statement with the default covariance structure (variance components, VC). Means are presented as least squares means and were separated by pairwise *t*-test (diff option of PROC MIXED).

RESULTS AND DISCUSSION

Diet information was presented inconsistently in the CC data set. The average composition of the diets used with the 2 types of CC systems was distinctly different.

Diets fed in studies using RUSITEC contained (DM basis) 92% OM (SD = 3; n = 206), 14% CP (SD = 5; n = 231), 44% NDF (SD = 20; n = 204), 19% starch (SD = 16; n = 12), and 4.0% ether extract (SD = 2.4; n = 111). Diets fed to non-RUSITEC fermentors contained, on average (DM basis), 93% OM (SD = 3; n = 356), 17% CP (SD = 9; n = 512), 34% NDF (SD = 12; n = 416), 34% starch (SD = 18; n = 93), and 3.7% ether extract (SD = 1.3; n = 184). Thus, diets fed to RUSITEC fermentors had higher NDF and lower starch content compared with those fed to non-RUSITEC fermentors. Some non-RUSITEC studies investigated high-concentrate diets, in which dietary NDF was as low as 8 to 10% of DM (Fu et al., 2001), or starch content was as high as 85 to 95% (Meng et al., 2000). The diets in the in vivo data set had an average content (DM basis) of 94% OM (SD = 8; n = 350), 16% CP (SD = 1.4; n = 352), and 32% NDF (SD = 9; n = 352).

Descriptive statistics of the data sets are shown in Table 1. The liquid dilution rate was notably lower [SEM = 0.003 (highest SEM published throughout the manuscript); $P < 0.001$] for the RUSITEC than the non-RUSITEC data set. Variability within data set was high for both CC systems. The average liquid dilution rate was rather low for the RUSITEC data compared with ruminal fluid passage rates in vivo (e.g., 0.16 h^{-1}, Hristov and Broderick, 1996; 0.083 h^{-1}, Eugène et al., 2004), and closer to ruminal solids (indigestible NDF) passage rates reported in vivo (Krizsan et al., 2010). Both liquid and particle passage rate can influence ruminal fermentation characteristics in CC systems. In most of the CC studies, the ratio between fluid and particle passage rate was much smaller than in vivo. Particle dilution rate in the RUSITEC is 0.021 h^{-1} or lower (Czerkawski and Breckenridge, 1977) and is comparable to the average indigestible NDF passage rate of 0.026 h^{-1} (n = 172 diets, DMI = 2.9% of BW) reported for growing and lactating cattle (Krizsan et al., 2010). The mean particle dilution rate in the non-RUSITEC data set was much faster (0.05 h^{-1}) than in vivo indigestible NDF passage rate. In contrast, as indicated earlier, fluid dilution rates in both CC data sets were markedly slower than in vivo (Hristov and Broderick, 1996; Eugène et al., 2004).

Both the level of dilution rates and the ratio between fluid and particle passage rates can influence ruminal fermentation characteristics. Increased liquid dilution rate in a dual-flow CC system (Eun et al., 2004) and RUSITEC (Martínez et al. 2009) increased ammonia N outflow from the fermentors, suggesting reduced efficiency of N utilization. In the study of Martínez et al. (2009) feed NAN flow decreased with increasing dilution rate. These findings are in contrast with in vivo data. In a meta-analysis of omasal flow data, ruminal CP balance (net ammonia absorption) was positively related to ruminal ammonia N concentration (Broderick et al., 2010), whereas the efficiency of microbial N synthesis increased with DMI and, consequently, dilution rate. Increasing intake would increase ruminal passage rate and reduce microbial retention time and, thus, increase microbial cell yield per unit of fermented energy by diluting maintenance expenditure (Russell et al., 1992).

In a dual-flow CC system, Eun et al. (2004) reported an average methane production of 3.5, 9.8, and 12.3% of digestible energy, with fluid dilution rates of 0.032, 0.063, and 0.125 h^{-1}, respectively. In addition to unrealistically large quantitative effect of dilution rate on methane production, the changes were opposite to those observed in vivo. Methane production as a proportion of energy intake decreased with increased feed intake and both fluid and particle dilution rates increased. For example, in the analysis of data from calorimetric studies, methane production decreased by 0.8 and 1.2% as a proportion of gross and digestible energy, respectively, per multiple of maintenance increase in feeding level (Yan et al., 2000). Martínez et al. (2009) reported increased VFA production with increased dilution rate in a RUSITEC system, in contrast to expected in vivo responses. Overall, the studies of Eun et al. (2004) and Martínez et al. (2009) demonstrated strong effects of dilution rate on many digestion and fermentation variables, but in most cases the responses were different from those expected in vivo.

Medium pH is usually maintained by continuous infusion of buffer in CC systems, and the average pH was above 6.0 for both RUSITEC and non-RUSITEC, similar to the in vivo data set (Table 1). Variability in pH was relatively low for both CC systems. Ammonia concentration reported for the CC studies was within the range of ruminal ammonia concentrations of the in vivo data set (SEM = 0.94; $P = 0.31$). The variability (CV, Table 1 and variance, Table 2), however, was particularly large for the non-RUSITEC data set. The average total VFA concentration for the RUSITEC and non-RUSITEC data sets was 67 and 80% (respectively) of the total VFA concentration in vivo (SEM = 4.9; $P < 0.001$) but within the range reported in a meta-analysis by Eugène et al. (2004) for various ruminant species (89 mM, SD = 18.2; n = 136). In contrast with our results, Martínez et al. (2010a) observed similar (or even higher) total VFA concentrations in the RUSITEC when directly compared with VFA concentrations in vivo in sheep fed the same diets. The average concentration of acetate was lower (SEM = 3.2; $P < 0.001$) for the CC data sets compared with in vivo; the molar proportion of acetate was 56, 59, and 62 mol/100 mol

Table 1. Descriptive statistics of the continuous-culture and in vivo data sets used in the meta-analysis[1]

Variable	n	Mean	SD	CV
RUSITEC continuous culture				
Liquid dilution rate,[2] h^{-1}	160	0.03	0.01	33.9
pH	288	6.84	0.25	3.7
Ammonia, m*M*	155	9.0	5.1	55.9
VFA concentration, m*M*				
Total	250	78.9	30.9	39.1
Acetate	211	43.9	18.1	41.3
Propionate	211	18.0	8.1	44.7
Butyrate	207	11.4	7.3	63.7
Isobutyrate	132	0.5	0.3	48.9
Valerate	143	3.6	2.4	68.8
Isovalerate	119	1.8	0.8	43.1
Acetate:propionate	251	2.7	1.0	35.7
Bacteria,[3] $\times 10^9$/mL	150	36.0	146.1	405.8
Protozoa, $\times 10^3$/mL	163	7.8	10.1	129.7
MPS,[4] g of N/kg of OM	71	24.7	8.9	35.9
Apparent digestibility, %				
DM	226	55.9	14.1	25.2
OM	177	52.2	13.9	26.7
CP	126	63.1	17.9	28.3
NDF	203	34.2	15.9	46.4
ADF	140	29.2	14.3	49.1
NSC	20	87.7	4.5	5.1
Non-RUSITEC continuous culture				
Liquid dilution rate, h^{-1}	566	0.09	0.05	58.1
Solid dilution rate, h^{-1}	309	0.05	0.01	19.6
pH	388	6.25	0.49	7.9
Ammonia, m*M*	469	7.8	6.5	83.5
VFA concentration, m*M*				
Total	425	93.8	36.3	38.7
Acetate	411	54.5	22.6	41.5
Propionate	412	23.6	13.8	58.4
Butyrate	412	11.3	6.2	54.3
Isobutyrate	280	0.8	1.1	136.8
Valerate	331	2.4	1.5	61.0
Isovalerate	274	1.6	1.7	107.2
Acetate:propionate	481	2.6	1.1	41.7
Bacteria,[3] $\times 10^9$/mL	37	64.2	60.1	93.6
Protozoa, $\times 10^3$/mL	61	121.6	176.3	145.0
MPS,[5] g of N/kg of OM	180	27.0	15.9	59.1
Apparent digestibility, %				
DM	210	51.1	15.5	30.3
OM	246	44.4	12.7	28.7
CP	314	61.8	37.6	60.8
NDF	308	45.5	17.2	37.7
ADF	245	45.3	17.7	39.0
NSC	203	80.2	10.9	13.6
In vivo data[6]				
pH	353	6.13	0.28	4.5
Ammonia, m*M*	366	7.6	3.2	42.5
VFA concentration, m*M*				
Total	366	116.9	19.8	26.3
Acetate	366	72.1	12.8	17.8
Propionate	366	26.0	7.2	27.7
Butyrate	366	13.2	3.7	27.7
Isobutyrate	366	1.0	0.3	27.7
Valerate	366	2.2	0.8	36.4
Isovalerate	366	1.7	0.5	30.0
Acetate:propionate	366	2.9	0.8	26.0
Total-tract apparent digestibility, %				
DM	245	67.5	6.0	8.9
OM	352	69.6	6.2	8.9

Continued

Table 1 (Continued). Descriptive statistics of the continuous-culture and in vivo data sets used in the meta-analysis[1]

Variable	n	Mean	SD	CV
CP	351	65.6	10.5	16.1
NDF	352	53.0	11.6	21.9

[1]From 180 references. Studies in the continuous-culture data set were classified into 2 categories: studies that used rumen simulation techniques (RUSITEC; Czerkawski and Breckenridge, 1977) and studies that used other continuous-culture system designs (non-RUSITEC).

[2]For the RUSITEC data, liquid dilution rate in most cases reported as dilution rate in the original publications.

[3]In most studies, represents fluid-associated bacteria.

[4]Microbial protein synthesis [g of N/kg of OM digested for RUSITEC (only 1 study, 4 treatment means, reported MPS as g of N/kg of OM truly digested).

[5]Microbial protein synthesis, reported as g of N/kg of OM truly digested.

[6]Data set from in vivo trials with lactating dairy cows (Hristov et al., 2001b, 2004a,b, 2005, 2008, 2009, 2010, 2011a,b,c; Hristov and Ropp, 2003; Foley et al., 2006; Vander Pol et al., 2008; Oelker et al., 2009; Agle et al., 2010a,b; Lee et al., 2011; Mathew et al., 2011; Tekippe et al., 2011; Reveneau et al., 2012a,b).

for the 3 data sets, respectively. The average concentration of propionate was considerably lower (SEM = 1.6; $P < 0.001$) for RUSITEC compared with in vivo. Butyrate concentrations were similar (SEM = 0.44; $P = 0.44$) between RUSITEC and non-RUSITEC CC, but lower (SEM = 0.53; $P < 0.001$) compared with in vivo (although the difference was smaller than for the other major VFA). Molar proportions of propionate were similar among data sets (23, 25, and 22 mol/100 mol, respectively). Thus, the difference in total VFA concentrations between CC and in vivo was primarily determined by lower acetate concentration (and propionate for RUSITEC) in the CC data sets. This difference, in turn, determined the generally lower (SEM = 0.08; $P < 0.001$) acetate:propionate ratio for CC compared with in vivo. Lower acetate:propionate ratios for RUSITEC than in vivo were reported in the Martínez et al. (2010a) study. Those authors also observed lower proportion of acetate and higher propionate in RUSITEC compared with in vivo, which is in line with results from this meta-analysis. In the current analysis, variability in VFA data was the highest (higher variance, Table 2) for the non-RUSITEC data set, followed by RUSITEC, and was the lowest for in vivo.

Bacterial counts tended to be lower (SEM = 11.9; $P = 0.07$) and more variable (higher CV) for RUSITEC compared with non-RUSITEC (Table 1). Lower bacterial counts for RUSITEC is consistent with the report of Meyer and Mackie (1986), who demonstrated that bacterial population inside nylon bags incubated in situ was lower than in the surrounding ruminal fluid. The non-RUSITEC data set had considerably higher (SEM = 22.6; $P < 0.001$) protozoal counts compared with RUSITEC. Average protozoal counts reported for the CC data sets were lower than in animals with normal rumen fauna or even feedlot cattle fed 90% grain diets (Hristov et al., 2001a). Out of the 227 studies (both RUSITEC and non-RUSITEC systems) that reported protozoal data, in 132 protozoal counts were either 0 or below 10×10^3/mL. The CC systems, however, are generally not designed to maintain protozoal populations (at least not for prolonged periods) and these results are not surprising. Martínez et al. (2010b) compared protozoal counts and diversity in RUSITEC with in vivo and reported that total protozoal counts were 0.2 to 1.2% of those in vivo and there was complete disappearance of *Isotrichidae* and *Ophryoscolecidae*, and lack of response to dietary change in the RUSITEC. Logically, these authors concluded that protozoal populations in the RUSITEC were not representative of those in the rumens of sheep fed the same diets.

Efficiency of microbial protein synthesis was reported in fewer studies in RUSITEC than in non-RUSITEC. In both cases, microbial protein synthesis per kilogram of OM truly digested (non-RUSITEC) or per kilogram of OM digested, as reported for most RUSITEC studies, was numerically similar (data were not evaluated statistically due to different units) between data sets and within the range reported by Clark et al. (1992), or used by the NRC (2001) dairy protein model (12 to 54 g of microbial N/kg of rumen-fermented OM). Bacterial (or protozoal) species composition was not reported in the vast majority of CC studies, and these data were not included in the analysis. Reports exist, however, showing a significant shift in bacterial populations in vitro and in CC systems. Slyter and Putnam (1967), for example, did not observe *Streptococcus bovis* in vivo, but the bacterium made up from 2 to 9% of the total strains examined on certain days of CC fermentation. Mansfield et al. (1995) reported a vast increase in amylolytic species as a proportion of the total viable count in vivo compared with CC (28.2 vs. 3.3%, respectively).

Table 2. Variance estimates for selected variables from continuous-culture[1] and in vivo[2] data sets[3]

Item	Estimated variance	SE[4]
Ruminal ammonia concentration, m*M*		
RUSITEC	8.0	1.0
Non-RUSITEC	22.2	1.6
In vivo	5.1	0.4
Ruminal VFA concentrations, m*M*		
Total VFA		
RUSITEC	907.7	81.9
Non-RUSITEC	1,311.5	90.1
In vivo	397.9	29.5
Acetate		
RUSITEC	305.9	30.0
Non-RUSITEC	507.5	35.5
In vivo	165.1	12.2
Propionate		
RUSITEC	58.9	5.8
Non-RUSITEC	185.5	12.9
In vivo	53.5	4.0
Butyrate		
RUSITEC	51.5	5.1
Non-RUSITEC	37.6	2.6
In vivo	13.5	1.0
Acetate:propionate		
RUSITEC	0.9	0.08
Non-RUSITEC	1.2	0.08
In vivo	0.6	0.04
Digestibility,[5] %		
DM		
RUSITEC	192.4	18.2
Non-RUSITEC	233.0	22.8
In vivo	36.9	3.4
OM		
RUSITEC	193.9	20.7
Non-RUSITEC	162.3	14.7
In vivo	38.1	2.9
NDF		
RUSITEC	254.4	25.5
Non-RUSITEC	290.8	23.6
In vivo	141.7	11.3
CP		
RUSITEC	324.2	41.4
Non-RUSITEC	1,414.9	113.1
In vivo	106.8	8.6

[1]From 180 references. Studies in the continuous-culture data set were classified into 2 categories: studies that used rumen simulation techniques (RUSITEC; Czerkawski and Breckenridge, 1977) and studies that used other continuous-culture system designs (non-RUSITEC).

[2]Data set from trials with lactating dairy cows (in vivo; Hristov et al., 2001b, 2004a,b, 2005, 2008, 2009, 2010, 2011a,b,c; Hristov and Ropp, 2003; Foley et al., 2006; Vander Pol et al., 2008; Oelker et al., 2009; Agle et al., 2010a,b; Lee et al., 2011; Mathew et al., 2011; Tekippe et al., 2011; Reveneau et al., 2012a,b).

[3]Data set n are as in Table 1.

[4]For all variables, test for significance of the variance (Pr > Z) was <0.001.

[5]For in vivo, total-tract apparent digestibility.

Simultaneously, the proportion of cellulolytic bacteria decreased from 5.4 (in vivo) to 1.7% (CC) of the total viable count. As a result, fiber degradability rates in CC were considerably lower than in vivo (Mansfield et al., 1995). Similar selective decrease in cellulolytic bacteria was reported for RUSITEC when directly compared with in vivo conditions (Martínez et al., 2010b).

Apparent digestibility of DM and particularly OM was lower (SEM = 2.66 and 1.05, respectively; $P < 0.001$) for non-RUSITEC compared with RUSITEC (Table 1). Average digestibilities of DM and OM were about 20 and 25% lower ($P < 0.001$) for the CC data sets compared with in vivo. Variability in DM and OM digestibility data was much lower in vivo than in CC data sets (both CV and variance, Table 2). Higher OM digestibility was reported in vivo for grass silage-based diets by Huhtanen et al. (2009), averaging 74% (CV = 5%). In both CC data sets, but particularly in non-RUSITEC, OM digestibility was numerically lower than DM digestibility, which was the opposite of the in vivo data. Examination of the non-RUSITEC data set showed that in some, but not all, studies, OM digestibility was considerably lower than DM digestibility (for example, Jones et al., 1998; Calsamiglia et al., 2002; Miller-Webster et al., 2002; Griswold et al., 2003). This phenomenon has been reported in vitro and explained by potentially greater ash digestibility (or solubility) in vitro compared with in vivo due to considerable endogenous ash secretion in vivo (McLeod and Minson, 1974). Others have suggested buffer salts contamination of the CC effluent as a cause for this anomaly (Miller-Webster et al., 2002). The reason for the large difference in OM digestibility between RUSITEC and non-RUSITEC, however, is not clear. Digestibility of CP was lower (SEM = 4.33; $P = 0.02$) for CC than in vivo; variability (CV and particularly the variance for the non-RUSITEC data) was larger for the CC data sets. Digestibility of NSC was high for both CC data sets, but statistically lower (SEM = 0.99; $P < 0.001$) for non-RUSITEC compared with RUSITEC.

Digestibility of NDF was, on average, 19 and 7 percentage units lower (SEM = 2.34; $P < 0.001$) for RUSITEC and non-RUSITEC data sets (respectively) compared with in vivo; NDF digestibility was also lower ($P < 0.001$) for RUSITEC compared with non-RUSITEC. Similarly, ruminal NDF degradability was up to 40% lower in RUSITEC than in vivo in the study by Martínez et al. (2010a). Variability (both CV and variance) was much greater for the CC data sets than in vivo. The particularly low NDF degradability reported with RUSITEC compared with non-RUSITEC, despite longer retention time, is perhaps partially related to the nylon bag method used to provide solid feed in the former system. Cellulolytic bacteria counts (Meyer and Mackie, 1986) and enzymatic activities (Huhtanen and Khalili, 1992; Huhtanen et al., 1998) are considerably lower within nylon bags incubated in the rumen

than in the surrounding ruminal contents, which can partially explain the lower NDF digestibility reported for RUSITEC compared with non-RUSITEC systems. Martínez et al. (2010a) also reported much lower enzyme activities in RUSITEC than in vivo, consistent with much lower NDF digestibility with the RUSITEC. Another important factor determining the lower NDF digestibility for the RUSITEC data set is the considerably higher average dietary NDF and lower starch content compared with the diets fed in the non-RUSITEC studies (44 vs. 34% and 19 vs. 34%, respectively). The average in vivo NDF digestibility was 63% (CV = 11%) in the Huhtanen et al. (2009) analysis, but average in vivo NDF digestibility as low as 46% (SD = 10.9) was reported for a data set of 237 lactating dairy cows (Weiss, 2010). Other meta-analyses reported average in vivo ruminal NDF degradability similar to the average NDF digestibility observed for the non-RUSITEC data set (Eugène et al., 2004). The relatively lower digestibility in the CC data sets compared with in vivo is a result of a combination of factors, including lack of postruminal digestion in the CC systems (Meyer et al., 1971). In addition, fibrolytic bacteria may be selectively lost in CC systems, which usually results in lower fiber digestibility rates compared with in vivo conditions (Mansfield et al., 1995; Martínez et al., 2010a,b) and much lower fibrolytic activities in the CC incubation medium compared with the rumen (Martínez et al., 2010b). This and the partial or complete loss of protozoa may explain the particularly lower NDF digestibility for CC compared with in vivo data sets in the current analysis.

CONCLUSIONS

This analysis demonstrated that CC systems are generally characterized with lower total VFA and acetate concentrations, extremely low counts or lack of ruminal protozoa, and lower-than-in vivo OM and NDF digestibilities. Digestibility data have to be interpreted with the understanding that CC systems are designed to simulate the rumen, not the total digestive tract. Digestibility of NDF was particularly low for the RUSITEC data, likely due to higher NDF and lower starch content of the diets fed, compared with non-RUSITEC systems, and lower fibrolytic enzyme activities inside the nylon bags used to provide solid feed in this system. Overall, variability was much greater for CC compared with in vivo experimental data, which could be partially attributed to variability in the design of the CC fermentors, variability in the ruminal inoculum, and perhaps more extreme experimental treatments than those in vivo.

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