Comparison of microbial fermentation data from dual-flow continuous culture system and omasal sampling technique: A meta-analytical approach

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ABSTRACT

Although the omasal sampling technique (OST) has been successfully used to estimate ruminal fermentation and nutrient flow, alternatives to invasive animal trials should be pursued and evaluated. The objective of this study was to evaluate carbohydrate and N metabolisms using a meta-analytical approach to compare 2 methods: dual-flow continuous culture system (DFCCS) and OST. To be included, studies needed to report diet chemical composition and report at least 1 of the dependent variables of interest. A total of 155 articles were included, in which 97 used the DFCCS and 58 used the OST. The independent variables used were dietary nonfiber carbohydrate concentration, neutral detergent fiber (NDF) degradability, true crude protein (CP) degradability, and efficiency of microbial protein synthesis (EMPS). In addition, 12 dependent variables were used. Statistical analyses were performed using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC). A random coefficients model was used considering study as a random effect and including the possibility of covariance between the slope and the intercept. The effect of method (DFCCS or OST) was included and tested in the estimates of the intercept, linear, and quadratic effects of the independent variable. There was no method effect when NDF degradability was regressed with total volatile fatty acids concentration, true CP degradability, and EMPS. Molar proportions of acetate and propionate were quadratically associated with NDF degradability. When NDF degradability was regressed with acetate and propionate there was a method effect, differing only in the intercept (β_0) estimate. True organic matter digestibility, bacterial N/total N, efficiency of N utilization, total volatile fatty acid concentration, and molar proportion of butyrate linearly increased as dietary nonfiber carbohydrate

concentration increased, and none of these variables were affected by method. Concentration of ammonia N had a linear and positive association with true CP degradability. This was the only variable that had a method effect when regressed with true CP degradability, differing only in the estimate of the intercept (β_0). As EMPS increased, efficiency of N utilization also increased, and it was affected by method. Overall, the majority of DFCCS responses were similar to OST. When a method effect was observed, it was mainly on the estimate of the intercept, demonstrating that the magnitude of these responses was different. However, the relationships between independent and dependent variables were similar across methods.

Key words: in vitro, nutrient flow, omasum, rumen

INTRODUCTION

Studies aiming to determine feed digestion, the effects of feed additives, or ruminal fermentation often require determination of ruminal fermentation end products and nutrient flow using cannulas fitted in the abomasum or duodenum (Ahvenjärvi et al., 2000). The omasal sampling technique (OST), described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000), is a well-accepted technique for assessing ruminal fermentation and nutrient flow. It has been successfully used for estimating nutrient flows and ruminal metabolism of nitrogen (N; Reynal et al., 2003), carbohydrates (Owens et al., 2008), fatty acids (Sterk et al., 2012), and minerals (Tuori et al., 2008). Although this technique provides valuable results and is considered adequate for estimating ruminal fermentation and nutrient flow, it is laborious and expensive. Therefore, alternative techniques capable of accurately simulating ruminal fermentation are warranted.

The dual-flow continuous culture system (DFCCS), described by Hoover et al. (1976), aims to simulate the continuous differential flows of liquid and solids from the rumen. It provides a closer response to in vivo fermentation than do closed-vessel incubations (Hoover et al., 1976). The system involves a long-term fermenta-

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tion, with periods varying from 8 d (Calsamiglia et al., 2002) to 11 d (Dai et al., 2019). It has been used mainly to evaluate the effect of feedstuffs and additives on fermentation, digestion, nutrient flow, and N metabolism in dairy (Brandao et al., 2018) and beef (Amaral et al., 2016) diets. One of the most important advantages of the DFCCS compared with other in vitro systems is the continuous removal of fermentation end products, which reduces issues with accumulating fermentation products, such as VFA and ammonia (NH_3), that can potentially inhibit fermentation. Additionally, the system allows for intense sampling, determination of degradation rates, and testing of feed additives that are in early developmental stages and not yet produced on a large scale, under a constant DMI and passage rate.

However, studies quantitatively comparing ruminal fermentation data originating from DFCCS with those originating from OST are still scarce. Hristov et al. (2012) compared the variability of data from continuous culture systems with in vivo data; however, that study included a wide variety of in vitro systems and compared them with in vivo total-tract digestibility, which can be different when compared with ruminal digestibility. Therefore, we hypothesized that ruminal carbohydrate and N metabolism have similar responses to the independent variables used in this study when estimated using DFCCS and OST. The objective of this study was to summarize the literature and evaluate carbohydrate and N metabolism using a meta-analytical approach to compare 2 methods, DFCCS and OST.

MATERIALS AND METHODS

Data Collection and Preparation

A search for relevant articles was made using PubMed, Science Direct, and Google Scholar databases as well as individual journal databases, such as *Journal of Dairy Science*, *Journal of Animal Science*, *Livestock*, *Animal Feed Science and Technology*, and *Animal* from summer 2018 to spring 2019. Data were collected from peer-reviewed articles published from 1985 to 2019, and 2 unpublished studies (DFCCS) from our research group were also included. The keywords used for the DFCCS data set were “dual-flow continuous culture system,” “in vitro,” “microbial fermentation,” and different combinations of these words. The keywords used for the OST data set were “microbial fermentation,” “nutrient flow,” “omasal sampling,” “omasum,” “rumen,” and “ruminal fermentation.” In each article evaluated, including meta-analysis and review articles, the reference list was also searched for relevant titles. Only articles written in English were considered in the present study.

As a search criterion for both DFCCS and OST, the study under evaluation needed to report the chemical composition of the diet (at least dietary CP and NDF) and at least 1 of the dependent variables of interest (Table 1). We used as independent variables dietary NFC concentration, NDF degradability, true CP degradability, and EMPS; the descriptive statistics are presented in Table 1 and Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17107>). The dependent and independent variables were selected with the aim of having a comprehensive set of fermentation responses related to ruminal N and carbohydrate metabolism. True CP degradability and EMPS were selected to represent N metabolism, and dietary NFC concentration and NDF degradability were used to represent carbohydrate metabolism. Therefore, to have a wider combination of variables used to develop the regressions, EMPS and degradability of NDF and true CP were used as independent and dependent variables.

To be included, the study needed to report individual treatment least squares means and standard error of the mean (SEM) or standard deviation. If the article did not report SEM but reported standard error of the means or standard deviation, then SEM was calculated according to Steel et al. (1997). We included only studies that performed omasal sampling through ruminal cannula, and no reticulum, abomasum, or duodenum sampling studies were included. Most of the DFCCS studies aimed to evaluate different feeds and additives. For the DFCCS data set, all studies used inoculum from dairy ($n = 368$ treatment means) or beef ($n = 87$ treatment means) cattle; therefore, studies using inocula from other ruminant species were not considered in the present study. This study considered only data from dual-flow continuous cultures system, and data from systems such as Rusitec or any other in vitro system were not included.

All studies using OST used changeover design, and out of 58 studies included, 5 used beef animals ($n = 13$ treatment means) and 53 used dairy cows ($n = 182$ treatment means). After a thorough review of the studies under consideration, the data were entered in the database, and the last step was to verify each data entry. A total of 153 peer-reviewed published articles and 2 unpublished studies (using DFCCS from our research group) were included, containing 650 treatment means (455 DFCCS and 195 OST). The mean number of observations per dietary treatment was 4.4 and ranged from 2 to 9. The complete list of studies included in this meta-analysis is presented in Supplemental File S1 (<https://doi.org/10.3168/jds.2019-17107>).

The solid passage rate for DSCCS ranged from 2 to 8%/h and averaged 5%/h, whereas the liquid passage

Table 1. Descriptive statistics of dependent (D) and independent (I) variables for the data set comparing microbial fermentation from dual-flow continuous culture system (DFCCS) and omasal sampling technique (OST) studies

Item, % unless otherwise stated	DFCCS	OST
NDF degradability (D, I)		
Mean	49.59	47.09
SD	16.47	13.70
N	308	176
Dietary NFC concentration (I)		
Mean	33.48	44.41
SD	14.64	4.83
N	170	73
True CP degradability (D, I)		
Mean	59.60	64.93
SD	16.54	9.50
N	275	110
EMPS, ¹ g/kg (D, I)		
Mean	28.18	28.79
SD	10.40	7.98
N	321	128
ENU ² (D)		
Mean	52.25	65.26
SD	19.76	10.92
N	317	136
True OM digestibility (D)		
Mean	57.51	66.46
SD	11.77	8.08
N	323	127
NH ₃ -N, ³ mg/dL (D)		
Mean	12.78	9.28
SD	6.28	4.33
N	355	174
Bacterial N/total N ⁴ (D)		
Mean	45.73	63.83
SD	12.42	9.34
N	256	98
NANMN/total N ⁵ (D)		
Mean	36.28	31.83
SD	18.14	8.28
N	256	98
Total VFA, mM (D)		
Mean	97.26	108.03
SD	27.65	18.29
N	395	176
Acetate (D)		
Mean	57.10	64.32
SD	9.44	3.79
N	395	176
Propionate (D)		
Mean	25.54	20.03
SD	8.56	3.19
N	395	176
Butyrate (D)		
Mean	11.72	11.77
SD	4.39	1.76
N	395	176

¹Efficiency of microbial protein synthesis (g of microbial N/kg of truly digested OM).

²Efficiency of nitrogen use, calculated according to Bach et al. (1999) for DFCCS and Brito et al. (2009) for OST.

³Ammonia nitrogen.

⁴Proportion of bacterial nitrogen over total nitrogen flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.

⁵Proportion of nonammonia nonmicrobial nitrogen over total nitrogen flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.

rate ranged from 4 to 12.5%/h and averaged 10%/h. Most experiments used artificial saliva, described by Weller and Pilgrim (1974), with addition of 0.4 g of urea/L to simulate N recycling. The average volume of the fermentation vessels was 1,209 mL, with a minimum of 700 mL and a maximum of 1,830 mL (SD = 289).

Efficiency of microbial protein synthesis (**EMPS**) was calculated as grams of microbial N divided by kilograms of OM truly digested in the rumen. When individual VFA were reported as a concentration, molar proportion was calculated using total VFA concentration. Efficiency of N use (**ENU**) was calculated according to Bach et al. (1999) for DFCCS studies and expressed as grams of bacterial N per gram of N intake (Brito et al., 2009) for OST. The NFC concentration was used only when the study reported it. The studies determined microbial N flow using ¹⁵N or purine derivatives. To allow comparison of N metabolism data between DFCCS and OST, bacterial N and nonammonia nonmicrobial N (**NANMN**) were divided by the total N flow and multiplied by 100. Therefore, these data are expressed in percentage of the total N flow.

Data Cleaning and Model Derivation Procedure

A preliminary graphical examination of the data was performed to identify the relationships between dependent and independent variables of interest as well as possible outliers (Sauvant et al., 2008). Data were weighted using the inverse of the standard error of the mean (Roman-Garcia et al., 2016) instead of the inverse of the squared standard error of the mean.

In this study, we used a data set of in vivo and in vitro studies; therefore, it was expected that the in vivo data set (OST) would have greater within-study variance than the in vitro data set (DFCCS). To prevent overweighting studies with very low SEM, SEM was truncated at one-fourth of the mean SEM among studies. The percentage of truncated SEM ranged from 6.19 to 15.63%, and this process was done for the DFCCS and OST data sets separately. After trimming the SEM, the weight factor was normalized within method (DFCCS and OST). Briefly, the weight factor was calculated and then divided by the mean of the respective distribution, resulting in a distribution with mean equal to 1. The normalization allowed equal weighting across methods, and dependent variables were weighted without the bias of the different methods used (Roman-Garcia et al., 2016).

All statistical analyses were performed using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC). A random coefficients model was used considering

study as a random effect and including the possibility of covariance between the slope and the intercept. The covariance parameter was considered nonzero when $P \leq 0.05$. Fifteen variance-covariance structures were tested, and the one that provided the smallest Akaike information criterion was used. Models were tested for linear and quadratic effects of the independent variable. Tables 2, 3, 4, and 5 present the coefficient of determination and residual mean squared error adjusted for the random effect of study, and the regression estimates are expressed as $\pm$ SEM. Further details regarding mixed model analysis to integrate quantitative results can be found in St-Pierre (2001).

The effect of method (DFCCS or OST) was always included and tested in the initial model, and it was tested on the estimates of the intercept, linear, and quadratic effects of the independent variable. To derive the equations and generate the reduced models, a backward elimination procedure was used to remove parameters that had $P > 0.05$. The initial model (complete) contained the intercept, linear, and quadratic terms of the independent variable and its interaction with the methods. Only 1 term was removed from the model at each step of the backward elimination; terms were sequentially removed starting with the greatest order. If a term with greater order was significant (i.e., quadratic), then the linear term was maintained in the model regardless of whether it was significant. Data from beef and dairy animals were included to provide a wider range of fermentation responses. To account for their different fermentation patterns, the effect of dairy or beef animal was included as a random effect in the model. Significance levels to fit the model assumed for fixed and random effects were $P \leq 0.05$. The effect of method was tested in NDF degradability, true CP degradability, and EMPS, and it was not significant for any of them ($P > 0.05$). It should be noted that this study was not intended to generate prediction models; instead, our objective was to understand and critically evaluate the functional form of the DFCCS fermentation responses compared with OST.

RESULTS

Independent Variables: NDF Degradability and Dietary NFC Concentration

Regressions using NDF degradability as an independent variable are presented in Table 2. True OM digestibility was quadratically associated with NDF degradability and was affected by method (Table 2). Only the estimates of intercept (β_0) were different, in which DFCCS had lower β_0 than OST (46.4% and 56.2%,

respectively). True CP degradability had a quadratic association with NDF degradability. Method did not influence true CP degradability.

Concentration of $\text{NH}_3\text{-N}$ linearly increased as NDF degradability increased, and it had a method effect (Figure 1A). This was the only variable that had the same estimate of β_0 (3.6 mg/dL) but different slope (0.15 and 0.07 mg/dL for DFCCS and OST, respectively). Bacterial N/total N was not associated with NDF degradability and had a method effect on the estimate of β_0 , in which the study-corrected mean was 44.4% for DFCCS and 64.1% for OST. The NANMN/total N linearly decreased as NDF degradability increased and had a method effect. The DFCCS had greater β_0 and smaller slope (β_1) than OST. The ENU was not associated with NDF degradability. The study-corrected mean of ENU was 55.4% for DFCCS and 65.2% for OST; therefore, ENU was 9.8 percentage points greater in OST than in DFCCS.

Total VFA concentration was positively and quadratically associated with NDF degradability (Table 2). Total VFA concentration was not affected by method. Molar proportions of acetate (Figure 2A) and propionate (Figure 2B) were quadratically related to NDF degradability and were affected by method, differing only in the intercepts. Acetate was positively associated with NDF degradability, and propionate was negatively associated with NDF degradability. Molar proportion of acetate had a greater intercept in studies using OST (52.7 mol/100 mol) than in studies using DFCCS (47.0 mol/100 mol). The opposite response was observed for molar proportion of propionate, in which the intercept was lower for OST studies (33.9 mol/100 mol) than for DFCCS studies (38.3 mol/100 mol). The NDF degradability was not associated with molar proportion of butyrate and did not have a method effect.

The regressions developed using dietary NFC concentration as an independent variable are presented in Table 3. As dietary NFC concentration increased, NDF degradability linearly decreased and was not affected by method. True OM digestibility and dietary NFC concentration were linearly and positively associated, and method did not affect this association. True CP degradability was quadratically associated with dietary NFC concentration, with a minimum point at 42% NFC, and it was not affected by method.

The $\text{NH}_3\text{-N}$ concentration linearly decreased as dietary NFC concentration increased and was not affected by method (Table 3). Bacterial N/total N had a linear and positive association with NFC concentration and was not affected by method. The NANMN/total N and EMPS were not affected by dietary NFC concentration or method, and their study-corrected means were 35.2

Table 2. Regressions ($\pm$ SEM) developed using NDF degradability (%) as an independent variable comparing microbial fermentation from dual-flow continuous culture system (DFCCS) and omasal sampling technique (OST) studies^{1,2}

Dependent variable, % unless otherwise stated	Method	P-value			P-value			P-value			R ²	AIC ³	RMSE ⁴	N
		Estimate	β_0	Method	Estimate	β_1	Method	Estimate	β_2	Method				
True OM digestibility	DFCCS	46.43 $\pm$ 2.33	0.001	0.001	0.017 $\pm$ 0.09	0.854	0.952	0.0037 $\pm$ 0.0009	0.001	0.225	0.94	2,038.7	2.51	253
	OST	56.24 $\pm$ 2.72	0.001	0.001	0.017 $\pm$ 0.09	0.854	0.952	0.0037 $\pm$ 0.0009	0.001	0.225	0.94	2,038.7	2.51	112
True CP degradability	—	48.60 $\pm$ 6.19	0.001	0.162	0.040 $\pm$ 0.25	0.290	0.268	0.0046 $\pm$ 0.0020	0.039	0.552	0.79	1,923.1	5.33	278
NH ₃ -N, ⁵ mg/dL	DFCCS	3.59 $\pm$ 2.39	0.001	0.106	0.153 $\pm$ 0.02	0.001	0.004	—	0.262	0.154	0.80	2,061.4	2.09	238
	OST	3.59 $\pm$ 2.39	0.001	0.106	0.079 $\pm$ 0.02	0.001	0.004	—	0.262	0.154	0.94	2,061.4	2.09	156
Bacterial N/total N ⁶	DFCCS	44.39 $\pm$ 1.43	0.001	0.001	—	0.771	0.764	—	0.682	0.099	0.82	2,052.8	5.24	206
	OST	64.08 $\pm$ 2.06	0.001	0.001	—	0.771	0.764	—	0.682	0.099	0.82	2,052.8	5.24	89
NANMIN/total N ⁷	DFCCS	58.20 $\pm$ 2.68	0.001	0.001	-0.461 $\pm$ 0.05	0.001	0.004	—	0.145	0.508	0.73	1,931.3	5.38	194
	OST	34.10 $\pm$ 5.51	0.001	0.001	-0.046 $\pm$ 0.13	0.001	0.004	—	0.145	0.508	0.73	1,931.3	5.38	81
EMPS, ⁸ g/kg	—	32.98 $\pm$ 2.01	0.039	0.213	-0.130 $\pm$ 0.03	0.001	0.331	—	0.976	0.707	0.83	2,236.0	2.98	383
ENU ⁹	DFCCS	55.42 $\pm$ 1.56	0.002	0.001	—	0.308	0.255	—	0.964	0.484	0.75	2,635.9	6.39	344
	OST	65.19 $\pm$ 2.16	0.002	0.001	—	0.308	0.255	—	0.964	0.484	0.75	2,635.9	6.39	344
Total VFA, mM	—	81.28 $\pm$ 9.48	0.001	0.101	1.028 $\pm$ 0.350	0.002	0.424	-0.0113 $\pm$ 0.0030	0.004	0.994	0.92	3,032.3	5.64	390
Acetate	DFCCS	46.96 $\pm$ 2.39	0.001	0.001	0.396 $\pm$ 0.111	0.001	0.132	-0.0028 $\pm$ 0.0010	0.028	0.236	0.83	2,327.8	2.73	249
	OST	52.71 $\pm$ 2.65	0.001	0.001	0.396 $\pm$ 0.111	0.001	0.132	-0.0028 $\pm$ 0.0010	0.028	0.236	0.83	2,327.8	2.73	163
Propionate	DFCCS	38.27 $\pm$ 2.4	0.001	0.037	-0.497 $\pm$ 0.110	0.002	0.143	0.0036 $\pm$ 0.0010	0.040	0.127	0.84	2,166.3	2.24	249
	OST	33.93 $\pm$ 2.61	0.001	0.037	-0.497 $\pm$ 0.110	0.002	0.143	0.0036 $\pm$ 0.0010	0.040	0.127	0.84	2,166.3	2.24	163
Butyrate	—	11.42 $\pm$ 0.28	0.001	0.699	—	0.130	0.852	—	0.164	0.545	0.75	1,629.4	1.39	417

¹ β_0 = intercept; β_1 = linear term; β_2 = quadratic term. Method = DFCCS or OST.²Probabilities ≤ 0.05 were considered significant.³Akaike information criterion.⁴Residual mean squared error, adjusted for the random study effect.⁵Ammonia N.⁶Proportion of bacterial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁷Proportion of nonammonia nonmicrobial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁸Efficiency of microbial protein synthesis (g of microbial N/kg of truly digested OM).⁹Efficiency of N use, calculated according to Bach et al. (1999) for DFCCS (g of microbial N/N available) and Brito et al. (2009) for OST (g of microbial N/N intake).

Table 3. Regressions ($\pm$ SEM) developed using dietary NFC concentration (%) as an independent variable comparing microbial fermentation from dual-flow continuous culture system (DFCCS) and omasal sampling technique (OST) studies^{1,2}

Dependent variable, % unless otherwise stated	P-value			P-value			P-value			R ²	RMSE ⁴	N
	Estimate β_0	β_0	Method	Estimate β_1	β_1	Method	Estimate β_2	β_2	Method			
NDF degradability	63.18 $\pm$ 3.89	0.001	0.983	-0.39 $\pm$ 0.09	0.001	0.312	—	0.820	0.921	1,370.8	4.21	195
True OM digestibility	50.10 $\pm$ 7.24	0.001	0.289	0.22 $\pm$ 0.07	0.043	0.260	—	0.671	0.595	689.0	3.07	154
True CP degradability	99.17 $\pm$ 9.08	0.001	0.672	-1.88 $\pm$ 0.58	0.002	0.672	0.022 $\pm$ 0.009	0.018	0.715	928.3	5.90	139
NH ₃ -N ⁵ , mg/dL	21.56 $\pm$ 1.58	0.001	0.713	-0.25 $\pm$ 0.04	0.001	0.546	—	0.700	0.912	849.9	1.72	174
Bacterial N/total N ⁶	41.11 $\pm$ 3.87	0.001	0.347	0.23 $\pm$ 0.09	0.018	0.712	—	0.537	0.449	1,091.8	5.83	160
NANMN/total N ⁷	35.20 $\pm$ 4.45	0.001	0.635	—	0.123	0.601	—	0.324	0.398	1,183.3	5.67	163
EMPS ⁸	26.96 $\pm$ 1.18	0.001	0.093	—	0.313	0.114	—	0.895	0.826	1,083.8	0.87	193
ENU ⁹	24.47 $\pm$ 4.20	0.001	0.233	0.81 $\pm$ 0.10	0.001	0.987	—	0.438	0.379	1,350.0	5.61	181
Total VFA, mM	50.18 $\pm$ 6.31	0.080	0.256	1.27 $\pm$ 0.14	0.001	0.331	—	0.848	0.921	1,690.5	7.05	208
Acetate												
DFCCS	68.57 $\pm$ 1.83	0.001	0.022	-0.24 $\pm$ 0.04	0.001	0.672	—	0.845	0.938	1,078.6	3.03	162
OST	73.31 $\pm$ 2.49	0.001	0.022	-0.24 $\pm$ 0.04	0.001	0.672	—	0.845	0.938	1,078.6	3.03	58
Propionate												
DFCCS	15.97 $\pm$ 1.66	0.001	0.013	0.22 $\pm$ 0.04	0.001	0.919	—	0.492	0.502	973.7	2.58	157
OST	12.31 $\pm$ 2.16	0.001	0.013	0.22 $\pm$ 0.04	0.001	0.919	—	0.492	0.502	973.7	2.58	58
Butyrate	8.066 $\pm$ 1.13	0.001	0.213	0.095 $\pm$ 0.02	0.001	0.449	—	0.636	0.980	692.0	1.62	218

¹ β_0 = intercept; β_1 = linear term; β_2 = quadratic term. Method = DFCCS or OST.²Probabilities ≤ 0.05 were considered significant.³Akaike information criterion.⁴Residual mean squared error; adjusted for the random study effect.⁵Ammonia N.⁶Proportion of bacterial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁷Proportion of nonammonia nonmicrobial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁸Efficiency of microbial protein synthesis (g of microbial N/kg of truly digested OM).⁹Efficiency of N use, calculated according to Bach et al. (1999) for DFCCS (g of microbial N/N available) and Brito et al. (2009) for OST (g of microbial N/N intake).

Table 4. Regressions (±SEM) developed using true CP degradability (%) as an independent variable comparing microbial fermentation from dual-flow continuous culture system (DFCCS) and omasal sampling technique (OST) studies.^{1,2}

Dependent variable, % unless otherwise stated	P-value			P-value			P-value			R ²	RMSE ⁴	N
	Estimate	β_0	Method	Estimate	β_1	Method	Estimate	β_2	Method			
True OM digestibility	35.18 ± 3.31	0.001	0.322	0.63 ± 0.11	0.001	0.605	-0.0033 ± 0.0008	0.001	0.184	1,895.5	2.31	424
NH ₃ -N, ⁵ mg/dL												
DFCCS	3.61 ± 1.89	0.932	0.001	0.13 ± 0.02	0.001	0.234	—	0.882	0.593	1,836.5	2.03	241
OST	-0.31 ± 2.18	0.932	0.001	0.13 ± 0.02	0.001	0.234	—	0.882	0.593	1,836.5	2.03	98
Bacterial N/total N ⁶	21.36 ± 4.48	0.001	0.751	0.48 ± 0.06	0.001	0.254	—	0.935	0.738	1,649.4	4.53	251
NANMN/total N ⁷	83.20 ± 1.89	0.001	0.555	-0.78 ± 0.02	0.001	0.494	—	0.337	0.202	1,414.2	2.99	265
EMPS ⁸	21.70 ± 1.59	0.001	0.151	0.091 ± 0.02	0.001	0.099	—	0.447	0.518	1,832.6	2.64	344
ENU ⁹	17.023 ± 3.67	0.001	0.495	0.68 ± 0.05	0.001	0.207	—	0.841	0.101	1,999.9	5.64	300
Total VFA, mM	87.81 ± 5.05	0.001	0.264	0.25 ± 0.07	0.001	0.133	—	0.961	0.456	2,724.3	7.66	336
Acetate	34.60 ± 5.84	0.001	0.154	0.72 ± 0.17	0.001	0.257	-0.0046 ± 0.0010	0.001	0.432	1,936.0	3.23	336
Propionate	53.93 ± 5.6	0.001	0.097	-0.87 ± 0.16	0.001	0.135	0.0057 ± 0.0010	0.001	0.339	1,804.6	2.83	334
Butyrate	11.40 ± 0.42	0.024	0.336	—	0.509	0.450	—	0.300	0.366	1,387.7	1.71	345

¹ β_0 = intercept; β_1 = linear term; β_2 = quadratic term. Method = DFCCS or OST.²Probabilities ≤ 0.05 were considered significant.³Akaike information criterion.⁴Residual mean squared error; adjusted for the random study effect.⁵Ammonia nitrogen.⁶Proportion of bacterial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁷Proportion nonammonia nonmicrobial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁸Efficiency of microbial protein synthesis (g of microbial N/kg of truly digested OM).⁹Efficiency of N use, calculated according to Bach et al. (1999) for DFCCS (g of microbial N/N available) and Brito et al. (2009) for OST (g of microbial N/N intake).

Table 5. Regressions ($\pm$ SEM) developed using efficiency of microbial protein synthesis (g of microbial N/kg of truly digested OM) as an independent variable comparing microbial fermentation from dual-flow continuous culture system (DFCCS) and onasal sampling technique (OST) studies^{1,2}

Dependent variable, % unless otherwise stated	Method	P-value			P-value			Estimate β_1	β_1	Estimate β_2	β_2	Method	AIC ³	R ²	RMSE ⁴	N
		Estimate β_0	β_0	Method	Estimate β_2	β_2	Method									
NH ₃ -N ⁵ , mg/dL	DFCCS	10.91 ± 2.31	0.175	0.001	—	0.703	0.112	—	0.766	0.358	0.74	2,056.1	0.74	2.51	263	
	OST	6.86 ± 2.41	0.175	0.001	—	0.703	0.112	—	0.766	0.358	0.74	2,056.1	0.74	2.51	113	
	DFCCS	23.33 ± 3.48	0.001	0.001	0.903 ± 0.11	0.001	0.08	—	0.249	0.513	0.86	2,146.7	0.86	4.69	234	
	OST	40.86 ± 4.06	0.001	0.001	0.903 ± 0.11	0.001	0.08	—	0.249	0.513	0.86	2,146.7	0.86	4.69	86	
NANMN/total N ⁷	—	45.081 ± 4.73	0.001	0.259	−0.39 ± 0.16	0.016	0.574	—	0.416	0.543	0.79	2,212.2	0.79	4.75	305	
	DFCCS	17.19 ± 5.62	0.026	0.001	1.46 ± 0.13	0.001	0.388	—	0.374	0.621	0.90	2,483.6	0.90	4.02	249	
ENU ⁸	OST	28.92 ± 5.82	0.026	0.001	1.46 ± 0.13	0.001	0.388	—	0.374	0.621	0.90	2,483.6	0.90	4.02	110	
	—	104.31 ± 3.25	0.005	0.486	—	0.391	0.828	—	0.261	0.194	0.89	3,059.5	0.89	7.40	381	
Total VFA, mM	DFCCS	59.11 ± 2.12	0.022	0.001	—	0.267	0.132	—	0.616	0.851	0.77	2,041.1	0.77	3.02	243	
	Acetate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Propionate	OST	63.056 ± 1.99	0.022	0.003	—	0.267	0.132	—	0.616	0.851	0.77	2,041.1	0.77	3.02	115	
	DFCCS	26.29 ± 2.29	0.026	0.003	—	0.554	0.313	—	0.784	0.948	0.79	2,309.1	0.79	3.19	282	
Butyrate	OST	21.49 ± 2.53	0.026	0.003	—	0.554	0.313	—	0.784	0.948	0.79	2,309.1	0.79	3.19	120	
	—	11.92 ± 0.50	0.027	0.369	—	0.825	0.948	—	0.372	0.370	0.72	1,673.1	0.72	1.55	408	

¹ β_0 = intercept; β_1 = linear term; β_2 = quadratic term.²Probabilities ≤ 0.05 were considered significant.³Akaike information criterion.⁴Residual mean squared error; adjusted for the random study effect.⁵Ammonia N.⁶Proportion of bacterial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁷Proportion of nonammonia nonmicrobial N over total N flow (omasal N flow for OST and effluent N flow for DFCCS), expressed as %.⁸Efficiency of N use, calculated according to Bach et al. (1999) for DFCCS (g of microbial N/N available) and Brito et al. (2009) for OST (g of microbial N/N intake).

Figure 1. Adjusted concentration of ammonia (NH_3) regressed with NDF degradability (A) and true CP degradability (B). Data were obtained from studies using dual-flow continuous culture ($\circ$) and their residuals ($\square$) and from omasal sampling technique ($\blacktriangle$) and its residuals ($\blacksquare$). Residuals (observed minus predicted) are represented in the x-axis 0-line.

and 27.0%, respectively. The ENU and dietary NFC concentration had a linear and positive association and were not affected by method.

Total VFA concentration, acetate, propionate, and butyrate were linearly associated with NFC concentration (Table 3). Total VFA concentration was not affected by method. Molar proportion of acetate (Figure 2C) was negatively associated with dietary NFC concentration and propionate (Figure 2D) was positively associated with dietary NFC concentration, and both had a method effect. The method affected only the estimate of β_0 , in which OST studies had a greater estimate of β_0 for acetate (73.3 mol/100 mol) and a lower estimate for propionate (12.3 mol/100 mol). Molar proportion of butyrate linearly increased as dietary NFC concentration increased and was not affected by method.

Independent Variables: CP Degradability and EMPS

The regressions developed using true CP degradability as an independent variable are presented in Table 4. Concentration of NH_3 -N was linearly and positively associated with true CP degradability (Figure 3B). This was the only dependent variable with a method effect when regressed with true CP degradability, differing only in the estimate of β_0 . Studies using OST had a lower estimate of β_0 (-0.3 mg/dL) than did those using DFCCS (3.6 mg/dL); however, they had the same estimate of β_1 .

Bacterial N/total N (Figure 3A) was linearly associated with true CP degradability and was not affected by method (Table 4). The NANMN/total N linearly decreased as true CP degradability increased and was

not affected by method (Figure 3B). The EMPS (Figure 3C), ENU (Figure 3D), and total VFA were linearly and positively associated with true CP degradability, and none of these variables were affected by method. Molar proportion of acetate quadratically increased as true CP degradability increased (Figure 2E), and molar proportion of propionate quadratically decreased (Figure 2F) as true CP degradability increased. Acetate had the maximum point at 78% of true CP degradability, and propionate had the minimum point at 76% of true CP degradability. Molar proportions of acetate, propionate, and butyrate were not affected by method.

Regressions developed using EMPS as an independent variable are presented in Table 5. Concentration of NH_3 -N was not associated with EMPS. The study-corrected mean value for DFCCS was 10.9 mg/dL and for OST was 6.7 mg/dL. Bacterial N/total N had a linear and positive association with EMPS. Method affected only the estimate of β_0 , and OST had greater bacterial N/total N than DFCCS.

The NANMN/total N was linearly and negatively associated with EMPS (Figure 3E). As EMPS increased, ENU linearly increased (Figure 3F) and had an effect of method on the estimate of the intercept (Table 5). Total VFA, acetate, propionate, and butyrate were not associated with EMPS. Total concentration of VFA was not affected by method, and the study-corrected mean value was 104.3 mM. Molar proportions of acetate and propionate were affected by method. The study-corrected mean for acetate was 59.1 and 63.1 mol/100 mol in DFCCS and OST, respectively, and the study-corrected mean for propionate was 26.3 and 21.5 mol/100 mol in DFCCS and OST studies, respectively.

Figure 2. Adjusted molar ruminal proportion of acetate and propionate regressed with NDF degradability (A and B), regressed with dietary NFC concentration (C and D), and regressed with true CP degradability (E and F). Data were obtained from studies using dual-flow continuous culture (○) and their residuals (□) and from omasal sampling technique (▲) and its residuals (■). Residuals (observed minus predicted) are represented in the x-axis 0-line.

DISCUSSION

Carbohydrates

True OM digestibility was positively associated with the 2 carbohydrate independent variables used in

the present study: NDF degradability (quadratically) and NFC concentration (linearly; Table 2). Changes in ruminal NDF degradability directly reflect on true ruminal OM digestibility considering that the rumen is the main site of fiber digestion (Broderick et al., 2010). Dietary NFC concentration was positively associated

with true OM digestibility, possibly due to its high ruminal fermentability. In a study including 87 studies from beef and dairy cattle and a wide variety of starch sources, Offner and Sauvant (2004) reported ruminal starch degradability of 71.0%. Therefore, increases in

dietary NFC concentration can positively affect true OM digestibility by providing a greater amount of highly fermentable carbohydrates.

The NDF degradability was used as an independent variable in Table 2, and it was used as a dependent

Figure 3. Adjusted proportion of bacterial N (A) and nonammonia nonmicrobial N (NANMN; B) from total N flow, efficiency of microbial protein synthesis (EMPS; C), and efficiency of N use (ENU; D) regressed with true CP degradability. Adjusted proportion of NANMN (E) and ENU (F) regressed with EMPS. Data were obtained from studies using dual-flow continuous culture (○) and their residuals (□) and from omasal sampling technique (▲) and its residuals (■). Residuals (observed minus predicted) are represented in the x-axis 0-line.

variable and regressed against dietary NFC concentration in Table 3. Typically, dietary NFC concentration increases at the expense of NDF, which results in a diet with lower fiber. In *in vivo* studies, the association of highly fermentable carbohydrates with lower dietary NDF results in a decrease in ruminal pH (Oba and Allen, 2003), which can affect fiber degradability. In DFCCS studies, pH is commonly controlled, suggesting that this negative association between NDF degradability and dietary NFC concentration is more sensitive to changes in substrate rather than only pH.

Concentration of VFA in the rumen is affected by several factors, including but not limited to rate and extent of OM digestion. Highly digestible feeds are degraded in the rumen, producing VFA as an end product (Russell et al., 1992). Therefore, in agreement with the digestibility responses, total VFA concentration increased in response to increases in NFC concentration and NDF degradability (Tables 3 and 2, respectively). Total VFA concentration was not affected by method when regressed with all independent variables used, suggesting that estimates of VFA concentration might be close when made using DFCCS and OST. The rate of VFA absorption by the ruminal wall can affect ruminal total VFA concentration (Hall et al., 2015); however, in DFCCS, there is no absorption and VFA are removed through digesta outflow. We speculate that the digesta outflow rate in DFCCS is similar to the rumen wall absorption rate and the rate of VFA that are washed out from rumen via passage rate, which may explain the lack of method effect on the estimates of VFA concentration.

Chemical structure of the protein and its interaction with carbohydrates are important factors that determine ruminal CP degradability (NRC, 2001). Part of the total CP in feeds is bound to the plant cell wall, and it can be slowly degraded or be of low biological availability (Sniffen et al., 1992). As plants mature, the contribution of this slowly degraded CP portion increases. Therefore, if a large portion of CP is bound to the plant cell wall (primarily lignin), CP degradability tends to decrease due to reduced microbial access to nitrogenous compounds. This may explain the observed positive association between true CP degradability and NDF degradability (Table 2). Therefore, this positive association between degradabilities of true CP and NDF is likely a reflection of a more digestible feed.

Molar proportion of butyrate usually does not change often and ranges from 10 to 20% (Bergman, 1990). In the present study, molar proportion of butyrate only responded to increments in dietary NFC concentration and had an overall study-corrected mean of 11.7%, which is within the literature values. Considering that

NFC encompasses starch and sugars, it has been shown in continuous culture (Vallimont et al., 2004) and *in vivo* (DeFrain et al., 2004) that increasing dietary NFC concentration might result in greater butyrate concentration. Additionally, the positive and linear response of butyrate to dietary NFC concentration is possibly associated with accumulation of lactate in the rumen due to the presence of large amounts of rapidly fermentable carbohydrates (Nagaraja and Titgemeyer, 2007). Increased concentration of butyrate has been observed in animals fed high-grain diets (Coe et al., 1999). Ruminal lactate metabolism can generate acetate, propionate, butyrate, and to a lesser extent caproate and valerate (Marounek et al., 1989). However, the primary end product varies depending on ruminal pH (Satter and Esdale, 1968): when the pH is acidic, butyrate is preferably produced from acetate (Satter and Esdale, 1968). It has been proposed that butyrate can be produced from acetate utilizing the 2 hydrogen atoms released by the oxidation of lactate to pyruvate; therefore, butyrate formation might work as a hydrogen sink (Esdale et al., 1968).

N Metabolism

Carbohydrates, primarily rapidly fermentable carbohydrates such as NFC, determine the energy available for microbial growth and microbial N yield (Schwab et al., 2006). Therefore, the negative and linear response of $\text{NH}_3\text{-N}$ to increases in dietary NFC concentration (Table 3) is likely associated with greater microbial protein synthesis, which results in lower $\text{NH}_3\text{-N}$ accumulation (Oba and Allen, 2003; Schwab et al., 2006). Indeed, bacterial N/total N increased as dietary NFC concentration increased (Table 3), whereas NANMN/total N was not associated with dietary NFC concentration. Interestingly, even though bacterial N/total N increased in response to NFC, EMPS was not associated with dietary NFC concentration. This suggests that yield of microbial protein increased due to abundance of substrate; however, the efficiency of converting truly digested OM into microbial N did not change. Bach et al. (2005) observed that bacterial N flow decreased as ruminal pH increased; however, EMPS was not associated with rumen pH, which commonly respond to changes in carbohydrate fermentability. Additionally, ruminal $\text{NH}_3\text{-N}$ was insensitive to EMPS (Table 5), and a similar response has been reported by Bach et al. (2005) and Oba and Allen (2003). When true CP degradability was used as a dependent variable (Tables 2 and 3), it was not affected by method, demonstrating that the estimate of true CP degradability using DFCCS is similar to that using OST.

Passage rate and digestion are competitive processes (Mertens, 1977) that affect ruminal fermentation. It is interesting to note that in DFCCS studies the passage rate is constant, whereas in OST studies it is variable and largely affected by intake and diet characteristics. Furthermore, it is possible that the continuous removal of fermentation end products reduces the issue with accumulation of products that can inhibit fermentation, resulting in responses similar to those observed in vivo.

Dependent Variables Affected by Method

When ENU was regressed with EMPS (Figure 3F), we observed a linear and positive association. However, when Bach et al. (2005) regressed these 2 variables, they reported a quadratic response, with a maximum point at 29 g of microbial N/kg of fermented OM and 69 g of microbial N/100 g of rumen available N. This different response may be attribute to several factors. First, Bach et al. (2005) included a smaller number of observations ($n = 136$) than the present study. Second, they had no studies with ENU <40%; however, even without points below 40% ENU, we would likely still have a linear association. Third, it is possible that some studies used in Bach et al. (2005) were also used in the present study; however, we have additional data from 2005 to 2019. Fourth, we included DFCCS and OST data in our analysis, whereas Bach et al. (2005) used only DFCCS. Finally, our equation has lower residual mean squared error (4.63 vs. 6.54), wider range of ENU, and greater coefficient of determination, suggesting a better fit of the model. Therefore, it is possible that our result is more robust than that reported by Bach et al. (2005).

In NRC (2001), EMPS was regressed against apparent ruminal N balance and had a negative linear relationship, with EMPS ranging approximately from 12 to 54 g of microbial N/kg of fermented OM, which is similar to our EMPS range (Figure 3F). This demonstrates that microbial use of N and energy utilization become uncoupled if available ruminal N is relatively high and fermented OM is low. This result supports the linear association observed in the present study when regressing ENU against EMPS. At low ENU, EMPS is also low, likely due to the low energy available. Also, if an abundance of energy is available and N is not limiting, the greater the energy available, the greater the efficiency of converting N into microbial N. The NRC (2001) assumes a fixed ENU of 85% and EMPS of approximately 21 g of microbial N/kg of fermented OM. In our data set when ENU is 85%, the projected EMPS is approximately 46 and 38 g of microbial N/kg of fermented OM for DFCCS and OST, respectively.

Even though NRC (2001) recommends a fixed EMPS, it acknowledged that EMPS is responsive to dietary manipulations. Therefore, EMPS provides valuable insights on microbial energy use, and ENU is an indicator of N use. These 2 variables are complementary, and their use in association is generally recommended.

Molar proportion of acetate reached the maximum point at approximately 70% NDF degradability, and molar proportion of propionate reached the minimum point at approximately 69% NDF degradability (Table 2), suggesting that the NDF degradability point that maximizes acetate is similar to the point that minimizes propionate (Figure 2A and B). Because the method has an effect only on the estimate of β_0 , the NDF degradability point that maximizes acetate and minimizes propionate is the same for both DFCCS and OST. Similarly, when molar proportions of propionate and acetate were regressed with dietary NFC concentration and EMPS (Tables 3 and 5), OST had a greater estimate of β_0 for acetate and lower propionate than did DFCCS.

Acetate was consistently lower and propionate was greater in DFCCS. Method affected the β_0 estimate of acetate and propionate in 6 out of the 8 regressions reported in Tables 2 to 5. This shift in the intercept demonstrates that even though the magnitude of the response was different, the functional relationship was maintained between methods. There is some evidence in the literature that molar proportion of acetate is lower in DFCCS due to a decrease in fibrolytic population (Mansfield et al., 1995). Our results are in agreement with Hristov et al. (2012), who also observed lower molar proportion of acetate in continuous culture studies compared with in vivo studies.

Concentration of $\text{NH}_3\text{-N}$ was related linearly and positively with true CP and NDF degradability (Figure 1). The study-corrected means of $\text{NH}_3\text{-N}$ concentration were 12.2 and 8.4 mg/dL in DFCCS and OST, respectively, resulting in a 30% difference. Increments in NDF degradability resulted in greater $\text{NH}_3\text{-N}$ accumulation in DFCCS; this was the only variable with nonsignificant β_0 but significant linear term (β_1) between methods (Table 2). This result can be partially explained by the fact that in DFCCS, no $\text{NH}_3\text{-N}$ absorption occurs through the wall and the only way out of the system is through overflow, whereas in vivo, $\text{NH}_3\text{-N}$ is absorbed through the portal-drained viscera, extracted by the liver, and converted to urea (Lapierre and Lobley, 2001). Urea is then excreted in the urine or recycled via saliva or other sections of the gastrointestinal tract (Gozho et al., 2008).

In an experiment studying the effect of increasing N intake on urea kinetics and recycling, Marini and

Van Amburgh (2003), using Holstein heifers, reported N recycling ranging from 29% in high-N diets to 83% in a low-N diet. Additionally, Lapierre and Lobley (2001) reported that in cattle, an average of 30 to 40% of the N intake is recycled and returned to the gut as urea. In DFCCS studies, urea is commonly added in the artificial saliva aiming to simulate N recycling (Hannah et al., 1986). This practice is important when low-CP diets are used, and similar to *in vivo*, the contribution of urea recycling is important to ensure microbial growth. Satter and Slyter (1974), using continuous culture fermenters, suggested a minimum of 5 mg/dL of ruminal $\text{NH}_3\text{-N}$ to ensure microbial growth. Therefore, in experiments in which the diet provides enough N, the addition of urea might result in a greater $\text{NH}_3\text{-N}$ input in the system than the microbial population is capable of converting into microbial N.

In our data set, dietary CP averaged 16.8% for DFCCS (minimum = 4.0, maximum = 28.7, SD = 3.2) and 16.3% for OST (minimum = 9.9, maximum = 23.8, SD = 2.1). Considering a hypothetical scenario of 0.4 g of urea/L added via saliva to the fermenters, a passage rate of 10%/h for liquids and 5%/h for solids, and a fermenter volume of 1,830 mL, a total of 1.728 g of urea (0.795 g of N) is added to each fermenter daily. Therefore, if the experimental diet was formulated containing 16.8% CP, the urea input by saliva represents approximately 28% of additional N input, which represents 28% of N recycling. This N recycling value is in agreement with *in vivo* data (Lapierre and Lobley, 2001; Marini and Van Amburgh, 2003).

In vivo, the ruminal N balance is represented by the N input coming from diet, rumen wall, saliva, and the endogenous fraction and the output from the rumen wall and flow to omasum. In DFCCS, the only way out of N is by overflow. Therefore, we speculate that the N added in the artificial saliva used in DFCCS should represent the balance (N recycling minus N output) instead of only the N recycling. The NRC (2001) assumes that approximately 15.2% of RDP is lost in the rumen at an apparent N balance of zero. Considering N recycling of approximately 28%, the balance would be approximately 13%. We speculate that by reducing urea in the artificial saliva to approximately 0.19 g/L, the ruminal $\text{NH}_3\text{-N}$ values obtained using DFCCS might be more closely related to OST. We also hypothesize that considering that N recycling is regulated by N intake (Marini and Van Amburgh, 2003), the amount of urea added in the artificial saliva needs to be adjusted according to factors such as CP level and extent of ruminal protein degradation. Depending on the diet fed to the fermenter, it is possible that this addition of 0.4 g of urea/L exceeds the ruminal microbial ability to metabolize NH_3 , resulting in accumulation, which might

explain the difference in $\text{NH}_3\text{-N}$ observed between the 2 methods. Therefore, studies adjusting the amount of urea added in the artificial saliva in DFCCS are warranted.

In summary, out of 41 regressions developed in the present study, method affected only 14 estimates of β_0 and 2 estimates of linear term (β_1). Because the majority of method effect was observed only in the estimate of the intercept, it is likely that treatment effects observed in DFCCS are maintained when tested *in vivo*; however, the magnitude of the response may be different. In those cases, results need to be interpreted cautiously when extrapolating DFCCS data to *in vivo*, especially regarding $\text{NH}_3\text{-N}$ concentration. Our results indicate that DFCCS provides valuable estimates of ruminal fermentation and that, overall, the functional responses observed in DFCCS studies are similar to those observed in OST studies.

CONCLUSIONS

This meta-analysis was performed to compare ruminal fermentation responses *in vitro* using the continuous cultures system with responses obtained from *in vivo* studies using OST. Overall, method affected OM digestibility and molar proportion of acetate and propionate; however, the difference was observed only in the estimates of intercept. Even though we observed a method effect for molar proportion of acetate and propionate, total VFA concentration was not affected by method. Method only affected NANMN/total N when regressed with NDF degradability, whereas bacterial N/total N was affected by method when regressed with NDF degradability and EMPS. Furthermore, true CP degradability and EMPS responses were not affected by method. Concentration of $\text{NH}_3\text{-N}$ was the only variable that had a method effect on estimate of intercept and slope, demonstrating that estimation of $\text{NH}_3\text{-N}$ using DFCCS needs further adjustments and that studies investigating this response are warranted. Therefore, even though we observed differences in the estimate of β_0 for some variables, in most cases the magnitude of the response was small, and the biological value of this difference is likely minimum. Most important, the functional responses to different dietary NFC concentrations, EMPS, and NDF and true CP degradability are overall maintained in DFCCS compared with OST.

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