Digestion and Nutrient Flow in Continuous Culture System and Animal Responses. Do They Match?

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Introduction

This paper is a summary of one of our recent studies, for more details and complete data, please see Brandão et al. (2020). Studies aiming to determine feed digestion, the effects of feed additives, or to study ruminal fermentation, often require determination of ruminal fermentation end-products and nutrient flow using a cannula 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 to assess ruminal fermentation and nutrient flow. It has been successfully used for estimating nutrient flows and ruminal metabolism of nitrogen (Reynal et al., 2003), carbohydrates (Owens et al., 2008), fatty acids (Sterk et al., 2012), and minerals (Tuori et al., 2006). Although this technique provides valuable results and is considered adequate to estimate 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**) was described by Hoover et al. (1976) aiming to simulate the continuous differential flows of liquid and solids from the rumen. It provides a closer response to in vivo fermentation than closed vessel incubations (Hoover et al., 1976). The system consists of a long-term fermentation, with periods varying from 8 (Calsamiglia et al., 2002) to 11 days (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 (Brandão 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 feed additives in early developmental stages that are not yet produced in large scale, under a constant dry matter intake and passage rate.

However, studies quantitatively comparing ruminal fermentation data originated from DFCCS to 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 different in vitro systems and compared them with in vivo total tract digestibility, which can be different when compared to ruminal digestibility. Therefore,

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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 the study was to summarize the literature and evaluate carbohydrate and N metabolism using a meta-analytical approach to compare two methods: DFCCS and OST.

Carbohydrates

True organic matter (**OM**) digestibility was positively associated with the two carbohydrate independent variables used in the present study: Neutral detergent fiber (**NDF**) degradability (quadratically), non-fiber carbohydrates (**NFC**) concentration (linearly; Brandão et al. 2020). 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. Offner and Sauvant (2004) reviewed 87 studies from beef and dairy cattle fed a wide variety of starch sources and reported ruminal starch degradability of 71.0%. Therefore, increases in dietary NFC concentration can positively impact true OM digestibility by providing a greater amount of highly fermentable carbohydrates.

The NDF degradability was used as an independent variable in Brandão et al. (2020) and it was also used as dependent variable and regressed against dietary NFC concentration in Brandão et al. (2020). 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 drop in ruminal pH (Oba and Allen, 2003), which can affect fiber degrabability. 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 volatile fatty acids (**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 end-product (Russell et al., 1992). Therefore, in agreement with the digestibility responses, total VFA concentration increased in response to increase in NFC concentration and NDF degradability (Brandão et al. 2020). 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 ruminal wall can affect ruminal total VFA concentration (Hall et al., 2015); however, in DFCCS there is no absorption and the VFA are removed through digesta outflow. We speculate that the digesta outflow rate in DFCCS is similar to the rumen hall absorption rate and the rate of VFA that is 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 crude protein (**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 plant cell wall (primarily lignin), CP degradability tends to decrease due to reduced microbial access to nitrogenous compound. This may explain the observed positive association between true CP degradability and NDF degradability (Brandão et al. 2020). Therefore, this positive association between degradability 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 also associated with accumulation of lactate in the rumen due to 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), in which 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 two hydrogens atoms released by the oxidation of lactate to pyruvate; therefore, butyrate formation might work as a hydrogen sink (Esdale et al., 1968).

Nitrogen

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 NH_3-N to increases in dietary NFC concentration (Brandão et al. 2020) is likely associated with greater microbial protein synthesis, which results in lower NH_3-N accumulation (Oba and Allen, 2003; Schwab et al., 2006). Indeed, bacterial N/total N increased as dietary NFC concentration increased (Brandão et al. 2020), while nonammonia nonmicrobial N relative to the total N was not associated with dietary NFC concentration. Interestingly, even though bacterial N/total N increased in response to NFC, efficiency of microbial protein synthesis 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 OM truly digested into microbial N did not change. Bach et al. (2005) observed that bacterial N flow decreased as ruminal pH increased; however,

efficiency of microbial protein synthesis was not associated with rumen pH which commonly respond to changes on carbohydrates fermentability. Additionally, ruminal NH_3-N was insensitive to efficiency of microbial protein synthesis (Brandão et al. 2020), and similar response has been reported by Bach et al. (2005) and Oba and Allen (2003). When true CP degradability was used as dependent variable (Brandão et al. 2020), it was not affected by method, demonstrating that estimate of true CP degradability using DFCCS is similar to OST.

Passage rate and digestion are two competitive process (Mertens, 1977) that affect ruminal fermentation, and it is interesting to note that in DFCCS studies passage rate is constant, while in OST 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 in vivo.

Dependent Variables Affected by Method

When efficiency of N utilization was regressed with efficiency of microbial protein synthesis (**Figure 3F**) we observed a linear and positive association. However, when Bach et al. (2005) regressed these two variables they reported a quadratic response, with a maximum point at 29 g microbial N/kg OM fermented and 69 g of microbial N/100 g rumen available N. This different response may be attribute to: 1) in their study they included a smaller number of observations (n = 136) than the present study; 2) They had no studies with efficiency of N utilization < 40% (However, even without points below 40% efficiency of N utilization, we would likely still have a linear association); 3) 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; and 4) we included DFCCS and OST data in our analysis, while Bach et al. (2005) used only DFCCS. Additionally, our equation has smaller root mean square of the error (4.63 versus 6.54), wider range of efficiency of N utilization, and greater R^2, suggesting a better fit of the model. Therefore, it is possible that our result is more robust than reported by Bach et al. (2005).

In the NRC (2001), efficiency of microbial protein synthesis was regressed against apparent ruminal N balance and had a negative linear relationship, with efficiency of microbial protein synthesis ranging approximately from 12 to 54 g of microbial N/kg of fermented OM, which is similar to our efficiency of microbial protein synthesis range (**Figure 3F**). That demonstrates that if available ruminal N is relativity high and fermented OM low, then microbial use of N and energy utilization becomes uncoupled. This result supports the linear association observed in the present study when regressing efficiency of N utilization against efficiency of microbial protein synthesis. At low efficiency of N utilization, efficiency of microbial protein synthesis is also low, likely due to the low energy available. Also, if there is abundance of energy 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 efficiency of N utilization of 85% and efficiency of microbial protein synthesis of approximately 21

g of microbial N/kg of fermented OM. In our dataset when efficiency of N utilization is 85%, the projected efficiency of microbial protein synthesis is approximately 46 g of microbial N/kg of fermented OM for DFCCS and 38 g of microbial N/kg of fermented OM for OST. Even though the NRC (2001) recommends a fixed efficiency of microbial protein synthesis, they acknowledged that efficiency of microbial protein synthesis is responsive to dietary manipulations. Therefore, efficiency of microbial protein synthesis provides valuable insights on microbial energy use, and efficiency of N utilization is an indicator of N use. These two 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 presented minimum point at approximately 69% NDF degradability (Brandão et al. 2020). Suggesting that the NDF degradability point that maximizes acetate is similar to the point that minimizes propionate (**Figure 1A and B**). Due to the method having an effect only on the estimate of $ß_0$, the NDF degradability point that maximizes acetate and minimize propionate is the same for DFCCS and OST. Similarly, when molar proportions of propionate and acetate were regressed with dietary NFC concentration and efficiency of microbial protein synthesis (Brandão et al. 2020), OST had greater estimate of $ß_0$ for acetate and lower propionate than DFCCS.

Acetate was consistently lower and propionate greater in DFCCS. Method affected the $ß_0$ estimate of acetate and propionate in six, out of the eight regressions reported in Brandão et al. (2020). This shift in the intercept demonstrates that even though the magnitude of the response was different, the functional relationship was maintained between methods. There are some evidences 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), that also observed lower molar proportion of acetate in continuous culture studies when compared to in vivo.

Concentration of NH_3-N was related linearly and positively with true CP and NDF degradability (**Figure 2**). The study corrected means of NH_3-N concentration were 12.2 mg/dL and 8.4 mg/dL in DFCCS and OST, respectively, resulting in a 30% difference. Increments in NDF degradability resulted in greater NH_3-N accumulation in DFFCS, and this was the only variable with non-significant $ß_0$ but significant $ß_1$ between methods (Brandão et al. 2020). This result can be partially explained by the fact that in a DFCCS, there is no NH_3-N absorption through the wall, and the only way out of the system is through overflow. While in vivo, NH_3-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 aiming to study the effect of increasing N intake on urea kinetics and recycling, Marini and Van Amburgh (2003) using Holstein heifers, reported N recycling ranging from 83% in a low N diet and 29% in high N diets. Additionally,

Lapierre and Lobley (2001) reported that in cattle, on average 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 NH_3-N to ensure microbial growth. Therefore, in experiments in which the diet provides enough N, the addition of urea might result in a greater NH_3-N input in the system than the microbial population is capable of converting into microbial N.

In our dataset, dietary CP averaged 16.8% for DFCCS (min = 4.0, max = 28.7, SD = 3.2) and 16.3% for OST (min = 9.9, max = 23.8, SD = 2.1). Considering a hypothetical scenario of 1) 0.4 g/L of urea added via saliva the fermenters; 2) passage rate of 10%/h liquid and 5%/h for solids; and 3) fermenter volume of 1,830 mL. A total of 1.728 g 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).

The ruminal nitrogen balance in vivo is represented by the N input via dietary, rumen wall, saliva, and endogenous and output from rumen wall and flow to omasum. In a 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 at an apparent N balance of zero, approximately 15.2% of RDP is lost in the rumen. 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 NH_3-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 extend of ruminal protein degradation. It is possible that depending on the diet fed to the fermenter, this addition of 0.4 g/L urea exceeds the ruminal microbial ability to metabolize NH_3, resulting in accumulation, which might explain the difference on NH_3-N observed between the two 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 only affected 14 estimates of $ß_0$ and 2 estimates of $ß_1$. Because the majority of method effects were only observed in the estimate of the intercept, it is likely that treatment effects observed in DFCCS are likely 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 NH_3-N concentration. Our results indicate that the DFCCS provides valuable estimates

of ruminal fermentation, and that overall, the functional responses observed in DFCCS studies are similar to OST.

Conclusions

This meta-analysis was performed aiming to compare ruminal fermentation responses in vitro using the continuous cultures system with responses obtained from in vivo studies using omasal sampling technique. Overall, method affected OM digestibility, 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 nonammonia nonmicrobial N relative to the total N when regressed with NDF degradability, while bacterial N/total N /total N was affected by method when regressed with NDF degradability and efficiency of microbial protein synthesis. Furthermore, true CP degradability and efficiency of microbial protein synthesis responses were not affected by method.

Concentration of NH_3-N was the only variable that had method effect on estimate of intercept and slope, demonstrating that estimation of NH_3-N using DFCCS needs further adjustments and studies investigating this response are warranted. Therefore, even though we observed differences in the estimate of $ß_0$ for some variables, in most cases the magnitude of the response was small, and the biological value of this difference is likely minimum. Most importantly, the functional responses to different dietary NFC concentration, efficiency of microbial protein synthesis, and NDF and true CP degradability are overall maintained in the DFCCS compared to OST.

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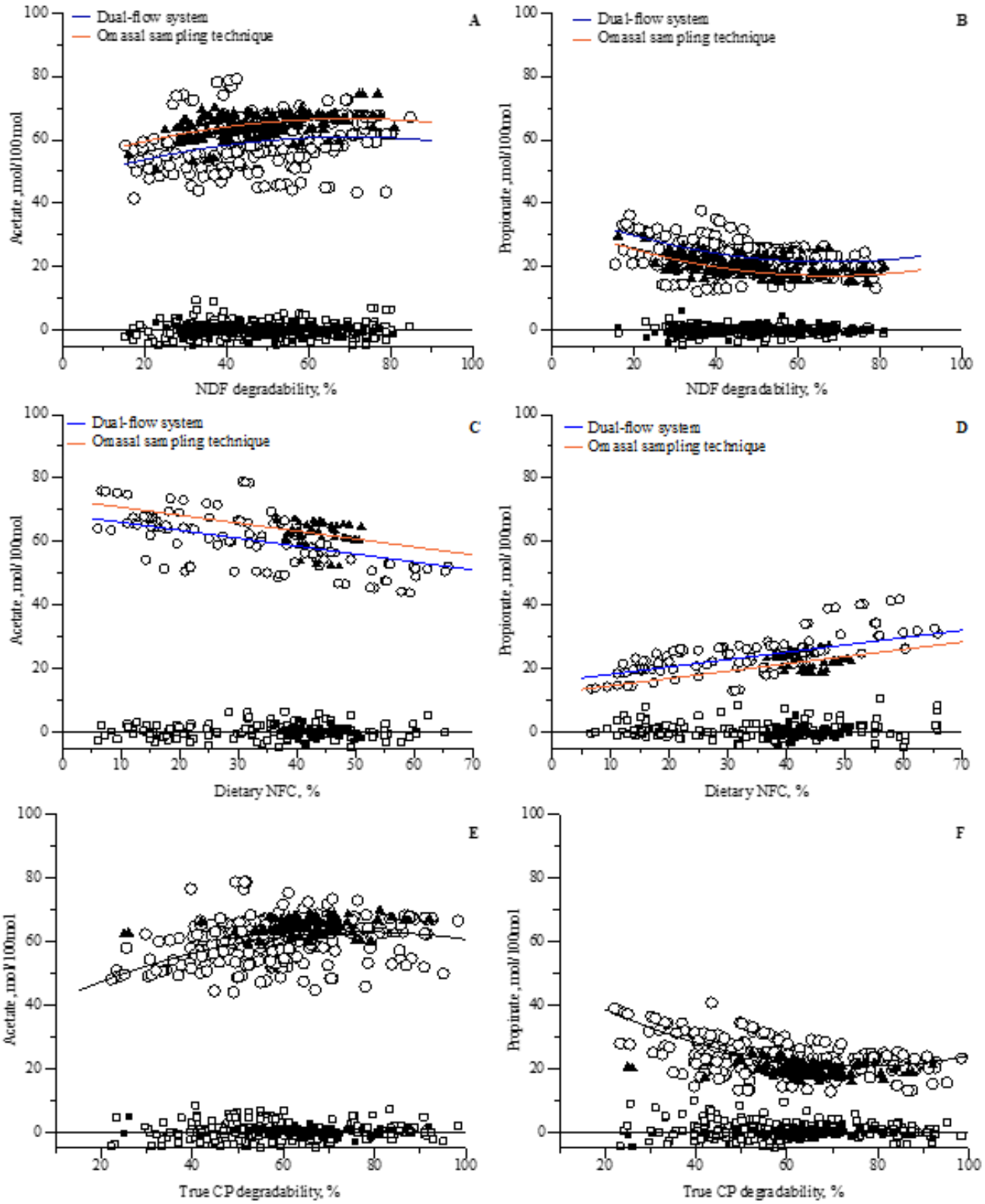

Figure 1. Adjusted ruminal molar proportion of acetate (A) and propionate (B) regressed with neutral detergent fiber degradability, regressed with dietary non-fiber carbohydrates

concentration (C and D), and regressed with true crude protein degradability (E and F). Data obtained from studies using dual-flow continuous culture (○) and their residuals (□); and from omasal sampling technique (▲) and its residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

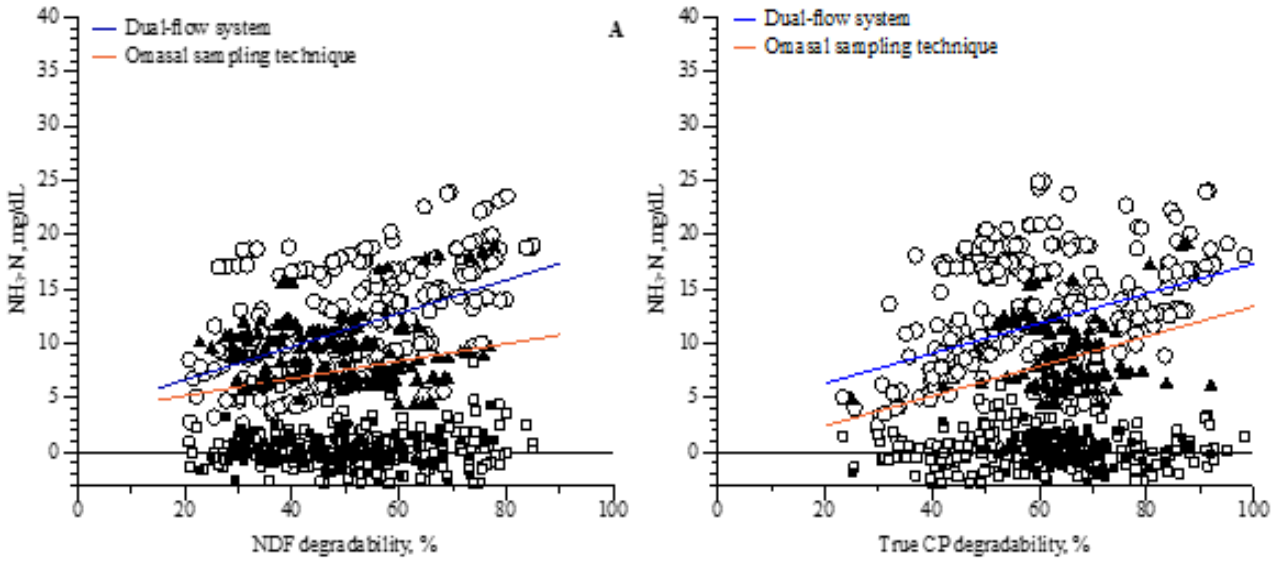

Figure 2. Adjusted concentration of ammonia regressed with neutral detergent fiber degradability (A) and true crude protein degradability (B). Data obtained from studies using dual-flow continuous culture (○) and their residuals (□); and from omasal sampling technique (▲) and its residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

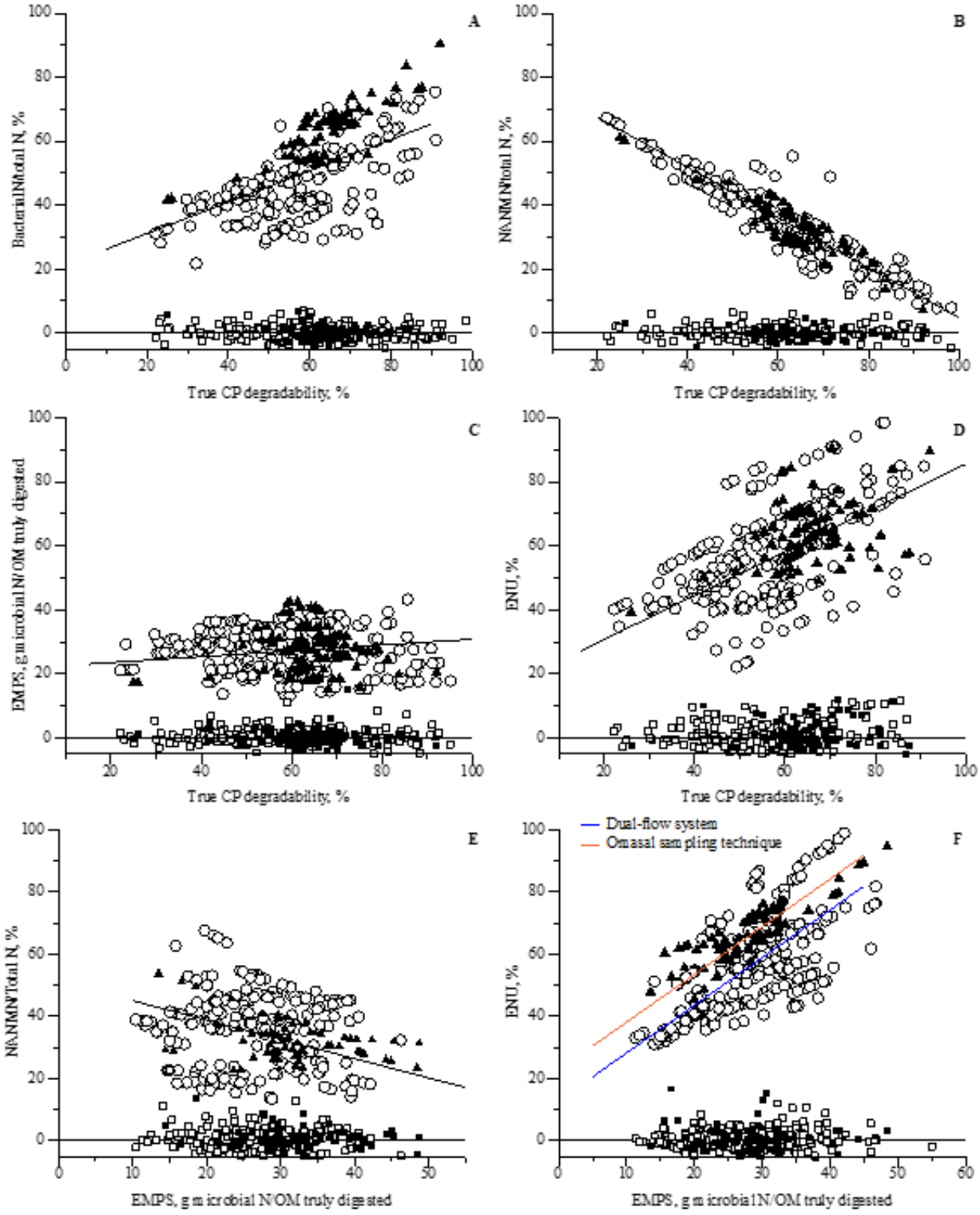

Figure 3. Adjusted proportion of bacterial nitrogen (A) and nonammonia nonmicrobial nitrogen (B) from total nitrogen flow, efficiency of microbial protein synthesis (C) and efficiency of

nitrogen use (D) regressed with true crude protein degradability. Adjusted proportion of nonammonia nonmicrobial nitrogen (E) and efficiency of nitrogen use (F) regressed with efficiency of microbial protein synthesis. Data obtained from studies using dual-flow continuous culture (○) and its residuals (□); and from omasal sampling technique (▲) and their residuals (■). Residuals (observed – predicted) are represented in the 0-line X axis.

SESSION NOTES