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Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation in vivo[1]

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ABSTRACT

Volatile fatty acid concentrations ([VFA], m*M*) have long been used to assess the effect of dietary treatments on ruminal fermentation in vivo. However, discrepancies in statistical results between [VFA] and VFA pool size (VFAmol) possibly related to ruminal digesta liquid amount (LIQ, kg) indicate potential issues with the use of [VFA]. We investigated relationships among [VFA], VFAmol, and LIQ measured 2 h postfeeding using individual lactating cow data (n = 175) from 7 separate feeding studies. Regression analyses were performed using mixed models with "study" as a discrete random variable. The mean across studies and average range of values within studies, respectively, were 151 and 75 for [VFA], 11.2 and 9.8 for VFAmol, 73.3 and 41.0 for LIQ, and 289 and 83 mmol/kg for rumen fluid osmolality. Liquid amount changed with VFAmol (3.76 VFAmol + 31.2; average within-study $R^2 = 0.69$), but the relationship was weak between [VFA] and LIQ (0.524 LIQ + 112.8; average within-study $R^2 = 0.12$). The relationship between LIQ and VFAmol was likely a function of the osmotic gradient between rumen liquid and blood. The VFA are a major ruminal solute; VFAmol amounts can affect water flux in the rumen as similar tonicities of rumen fluid and blood are maintained. This also has a damping effect on ruminal solute concentration, creating the weak relationship between [VFA] and LIQ. Within studies, similar [VFA] were found in LIQ differing by 30 kg or more. The difference between minimum and maximum LIQ within cow within study was 12.7 kg (standard deviation = 7.1), so inclusion of "cow" in analyses did not correct for the variation in LIQ. To allow valid comparisons of experimental treatments, responses must be on an equivalent basis; concentrations in different LIQ are not on an equivalent basis and so are not valid to use for comparing treatment effects. The [VFA] changed with VFAmol (5.80 VFAmol + 86.3; average within-study $R^2 = 0.56$). However, the ratio of [VFA] to VFAmol ranged from 9.0 to 24.1 as a function of 1,000/LIQ; this reflects the inherent calculated relationship among the variables. The varying relationship of [VFA] to VFAmol further indicates that [VFA] is not an appropriate measure to evaluate the progress or effect of treatments on ruminal fermentation. Predictions of LIQ and VFAmol using cow and ruminal measures were insufficiently precise to be used in research. Previously drawn conclusions based on [VFA] need to be reevaluated, and alternate evaluations for in vivo ruminal fermentation are needed.

Key words: volatile fatty acid concentration, ruminal fermentation, rumen digesta, regression analysis

INTRODUCTION

A fundamental principle of comparing experimental treatments is that all data are on an equivalent basis. It is for this reason that packed cell volume may be used as a covariate for evaluation of plasma values, or why dietary composition is evaluated on a dry matter basis, rather than "as fed." In the laboratory, concentrations of analytes detected in diluted samples are only useful if the dilution factor or the weight of sample and final dilution volume are known.

Ruminal concentrations of VFA ([**VFA**]) and other analytes have been used to describe the progress of ruminal fermentation in vivo (Phillipson, 1942) and the effects of dietary treatments since at least the 1940s. Rumen [VFA] are still commonly used to make statistical inferences regarding the effect of treatments on in vivo ruminal fermentation (e.g., Hall et al., 2010; 37 publications in the *Journal of Dairy Science* and *Journal of Animal Science* in 2012, alone). The authors may or may not be specific regarding what effect on fermentation is represented by the change in concentration, but the common implication is that treatments relatively increased or decreased fermentation of OM in

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the rumen and formation of microbial products. This use of [VFA] to evaluate treatment effects implicitly assumes that all other nontreatment factors that affect concentration are equivalent among treatments. Such nontreatment factors can include absorption and passage of VFA, as well as the amount of ruminal liquid into which the mass of VFA is diluted. However, a recent study reported altered interpretation of treatment effects when ruminal data were evaluated as [VFA] or as pool size or moles of VFA (**VFAmol**; Hall, 2013). The differences between the response variables were apparently due to variation in rumen liquid amounts. Diluted into differing liquid amounts, [VFA] measurements were not on the equivalent liquid amount basis needed to compare treatments, whereas VFAmol or VFA molar percentages are unaffected by digesta liquid amount.

For [VFA] to be useful for assessing treatment effects and rumen function, measures of rumen liquid volume are needed. However, use of ruminal liquid markers is indirect, they need time to equilibrate (Teeter and Owens, 1983) and will not differentiate between marker passage from the rumen and dilution of marker due to influx of liquid into the rumen. Manual rumen emptying to obtain a direct measurement is laborious and cannot be done repeatedly in short time frames without disturbing rumen function (D. K. Combs, University of Wisconsin, personal communication). Two key questions arise regarding rumen liquid amount: what factors affect it and can it be estimated in some relatively noninvasive way? In addition to direct water consumption or passage of liquid to the abomasum, ruminal liquid amount is driven primarily by the moles of soluble materials present and the osmotic gradient between rumen fluid and blood (Dobson, 1984). If rumen fluid is relatively hypotonic to blood, water is absorbed out of the rumen, whereas if rumen fluid is relatively hypertonic, water is absorbed across the epithelium and into the rumen (Tabaru et al., 1990). Ruminal entry or exit of water has the effect of diluting or concentrating ruminal solutes, and so affects ruminal concentration values. Solutes that affect rumen fluid osmolality include soluble minerals, organic acids, ammonia, AA, and soluble carbohydrates, with VFA representing 30 to 40% of the total (Warner and Stacy, 1965; Girard et al., 2009).

The objectives of this study were to investigate the variation in and relationships among [VFA], VFAmol, and rumen digesta liquid amount (**LIQ**) using individual lactating cow data measured in 7 separate feeding trials. Additionally, ruminal analyte concentrations and animal measures were evaluated to determine their potential for predicting LIQ and VFAmol without requiring rumen emptying or use of markers.

MATERIALS AND METHODS

Experimental Design

A data set of individual cow observations (n = 175) from 7 separate lactating cow feeding trials performed with ruminally cannulated cows was used in the evaluations. Feeding trials were included if they provided data on rumen liquid amount and concentrations of VFA determined at the same time of day. The trials were performed in different years with different treatment diets (Table 1) and largely used different animals. The experimental diets were corn silage- and alfalfa silage-based, with the exception of study G, in which grass hay or pasture was offered. Six of the studies were conducted at the US Dairy Forage Research Center farm (Prairie du Sac, WI) and 1 was conducted at Purdue University (West Lafayette, IN). All animals were maintained under protocols approved by the Institutional Animal Care and Use Committees of the University of Wisconsin or of Purdue University.

Rumen emptying was performed 2 h after feeding for each cow on 1 d of each period, or after morning milking for cows in study G. Rumen contents were manually removed via the rumen cannula and placed

Table 1. Lactating cow study descriptions

Study	Description
A	Calcium oxide-treated corn stover substituted for corn grain at 0, 4, 8, and 12% of diet DM. Eight cows, 4 × 4 Latin square, four 21-d periods.
B	Starch source × rumen protein degradability, 2 × 2 factorial. Eight cows, incomplete Latin square, three 21-d periods.
C	Physically effective fiber (chopped wheat straw, ensiled chopped corn stover) × starch source, 2 × 2 factorial. Eight cows, incomplete Latin square, three 21-d periods.
D	Physically effective fiber (chopped grass hay, ensiled chopped corn stover) × starch source, 2 × 2 factorial. Eight cows, incomplete Latin square, three 21-d periods.
E	Physically effective fiber (alfalfa stems, ensiled chopped corn stover) × starch source, 2 × 2 factorial. Eight cows, incomplete Latin square, three 21-d periods.
F	Potassium carbonate supplementation at 0, 1.6, and 3.2% of diet DM. Nine cows, 3 × 3 Latin square, three 18-d periods.
G	Cool season grass pasture grazing or hay feeding. Eight cows, covariate period followed by 2-period switchback design, three 21-d periods.