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The plasma free amino acid dose-response technique: A proposed methodology for determining lysine relative bioavailability of rumen-protected lysine supplements

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ABSTRACT

Estimates of Lys bioavailability of rumen-protected Lys (RP-Lys) supplements are often obtained using in vitro or 2-step in situ techniques, with little to no data determining efficacy and bioavailability in vivo. The objective of this study was to further evaluate and refine the use of the plasma free AA dose-response technique as a method for determining Lys relative bioavailability of RP-Lys supplements. Thirteen dose-response Latin square studies using 87 lactating, ruminally cannulated multiparous Holstein cows (days in milk from 55 to 315 and milk yield from 12 to 62 kg/d at the start of the studies) were conducted to measure the relative bioavailability of RP-Lys supplements. Intestinal (1 study) and abomasal (12 studies) infusions of Lys ranged from 0 to 84 g/d, and experimental periods ranged from 4 to 21 d. Basal diets were formulated to be adequate in metabolizable Met, but varied in predicted metabolizable Lys (5.04 to 6.81% of metabolizable protein). One to 4 daily blood samples were taken from the coccygeal vessels for 1 to 3 consecutive days in each period. Plasma Lys concentration in cows assigned to the control treatment (0 g/d Lys) ranged from 1.83 to 5.21% of total plasma AA, whereas that from cows duodenally or abomasally infused with Lys ranged from 2.53 to 7.51% of total plasma AA. Results from studies involving more than 2 amounts of infused Lys confirmed linearity of response. The following variables were regressed against the plasma Lys dose-response slopes generated from the Lys infusion treatments to examine their effects on the magnitude of the slopes: plasma Lys concentration of the control diet, plasma Lys concentration at the greatest amount of infused Lys, net energy of lactation and metabolizable protein balances, metabolizable protein supply, days in milk, milk yield, milk concentrations of fat, true protein, and lactose, milk true protein yield, and dry matter intake. The variable having the greatest effect on the magnitude of the dose-response slope was the plasma Lys concentration at the greatest amount infused. The relative bioavailability of evaluated RP-Lys supplements using the plasma free AA dose-response technique ranged from 5 to 87%. It was concluded that plasma free Lys increases in a linear fashion to increasing amounts of absorbed Lys and that the dose-response technique is an appropriate technique for evaluating RP-Lys supplements.
Key words: dairy cow, dose-response, lysine, relative bioavailability

INTRODUCTION

Lysine and Met are the 2 most limiting AA in typical North American dairy diets (NRC, 2001). Increasing Lys in MP to more optimal concentrations by postruminal infusion (King et al., 1991; Schwab et al., 1992) or by feeding a rumen-protected Lys (**RP-Lys**) supplement (Robinson et al., 2011; Lee et al., 2012) increased milk protein yield. Several RP-Lys supplements are available for the purpose of formulating rations for increased predicted concentrations of Lys in MP. Availability, variability in nutritional composition, digestibility, and cost of blood and fish meals have made RP-Lys supplements an attractive alternative, or a partial substitute, to these 2 RUP, Lys-rich supplemental protein sources. The RP-Lys supplements currently available on the market differ not only in terms of encapsulation technology, but also in size, density, and Lys concentration. Limited research indicates that the apparent availability of Lys to ruminants from RP-Lys supplements also varies widely (Robinson et al., 2011; Wu et al., 2012; Larson et al., 2015), making reliable estimates of Lys bioavailability essential when considering the use of these products. Although most suppliers provide estimates of Lys bioavailability for their RP-Lys supplements, these bioavailability values were not obtained using standardized techniques or methodologies. Without a standardized procedure

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for differentiating RP-Lys supplements under feeding conditions closer to commercial practices, producers and industry personnel have no reliable information to make decisions about which RP-Lys supplement to use based on price and amount of absorbable Lys.

The in situ technique that was developed to measure digestion of feed DM and its constituent organic fractions in the rumen and small intestine has been used to evaluate rumen-protected AA (**RP-AA**) supplements (Overton et al., 1996; Berthiaume et al., 2000; Koenig and Rode, 2001). The in situ technique has the advantage of obtaining independent estimates of ruminal escape and intestinal absorption of an AA for an RP-AA supplement from which an overall estimate of bioavailability can be calculated. Even though feedstuffs are subjected to ruminal fermentation and intestinal digestion with the in situ technique, several limitations exist, including (1) the absence of chewing and rumination effects; (2) potential discrepancy between nutrient disappearance from a bag and nutrient digestibility or absorption (Reynal et al., 2007); and (3) low reliability when testing fine or soluble supplements because they may be washed out of the bag (NRC, 2001; Robert, 2004, Kononoff et al., 2007). Ross et al. (2013) developed an in vitro assay for isolating the RUP fraction of feedstuffs and measuring its intestinal digestibility. This in vitro technique has been adapted for obtaining rates of ruminal degradation and estimates of intestinal digestibility of RP-AA supplements. Whereas the in vitro technique from Ross et al. (2013) has some decided advantages over the in situ method, the effect of exposing the RP-AA supplement to the ration before consumption, which has been shown to affect the integrity of some RP-Lys supplements (Ji et al., 2016) as well as chewing and rumination effects on bioavailability of AA from RP-AA, are eliminated. The Ross et al. (2013) in vitro technique has also not been published in a peer-reviewed journal, whereas the other techniques have.

In vivo techniques that have been used to determine AA bioavailability include the production response approach (Schwab et al., 2001), the area-under-the-curve method (Graulet et al., 2005), and the plasma free AA dose-response technique (Rulquin and Kowalczyk, 2003; Borucki Castro et al., 2008; Hanigan et al., 2009). The major limitation for the production response approach is being able to ensure a deficiency of the AA, in all animals, over the entire range of the treatment dosages that are used so that linearity in responses (e.g., concentration or yield of milk protein) are observed. For the area-under-the-curve technique, animals receive a single pulse dose of the RP-AA in amounts not normally encountered by the ruminal microbiota, which may limit supplement degradation. For studies where the plasma dose-response technique has been used (Rulquin and Kowalczyk, 2003; Borucki Castro et al., 2008; Hanigan et al., 2009), the limitation was that not all treatments (i.e., infused and fed doses of AA) were tested simultaneously within the same Latin square, thereby ignoring potential animal variation.

The plasma free Lys dose-response technique that we propose is a refinement of the plasma AA dose-response developed by Rulquin and Kowalczyk (2003). The technique relies on a positive linear relationship between incremental amounts of infused or fed Lys and plasma Lys concentration. In fact, a linear relationship between incremental doses of infused Lys into the omasum, abomasum, or duodenum, and plasma Lys concentration has been reported (Rulquin and Kowalczyk, 2003; Borucki Castro et al., 2008; Hanigan et al., 2009). We hypothesized that increased plasma Lys concentration would reflect increased net absorption of Lys, and that changes in plasma Lys concentration could be used to calculate reliable estimates of Lys relative bioavailability (**RBV**) in RP-Lys supplements. The objective of our study was to further evaluate and refine the use of the plasma free AA dose-response technique as a method for determining Lys RBV of RP-Lys supplements, which was achieved by (1) confirming linearity of the response between incremental amounts of infused Lys and plasma Lys concentration; (2) examining cow-to-cow variation in plasma Lys concentration in response to increasing amounts of absorbed Lys and the consequent effect on RBV of Lys in RP-Lys supplements; (3) examining animal factors, covariate analysis, and carryover effects among other variables; and (4) comparing the RBV of RP-Lys supplements using the plasma free Lys dose-response technique.

MATERIALS AND METHODS

All procedures related to animal care were conducted with approval of the University of New Hampshire Institutional Animal Care and Use Committee (protocols no. 070902, 091201, 110502, 111103, 121201, and 130904). All studies were conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center (Durham, NH). Studies were conducted in 2001 (study 1), 2008 (study 2), 2010 (studies 3 and 4), 2011 (studies 5 and 6), 2012 (study 7), 2013 (studies 8 to 11), and 2014 (studies 12 and 13).

Experimental Design and Treatments

The data set used herein came from 13 Latin square studies that were conducted at the University of New Hampshire to calculate the RBV of Lys from 27 RP-Lys supplements. It is important to note that some of the