



J. Dairy Sci. 100:1–19  
<https://doi.org/10.3168/jds.2016-11813>  
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## Relationships between postruminal casein infusion and milk production, and concentrations of plasma amino acids and blood urea in dairy cows: A multilevel mixed-effects meta-analysis

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### ABSTRACT

The relationships between postruminal casein infusion and production variables and concentrations of plasma AA and blood urea were evaluated using multi-level mixed-effects models derived from literature data collected in dairy cows. The data set contained 147 treatment means [i.e., 66 controls (CTL) and 81 casein-infused (CAS) means]. Each CAS mean was paired with its corresponding CTL mean to create 81 mean differences (CAS minus CTL), which were analyzed as absolute and percentage-based units (i.e., percentage increase or decrease in CAS relative to CTL). The primary variable of interest was the difference in estimated metabolizable protein (MP) supply ( $\Delta$ MP) between CAS and CTL. The other explanatory variables were based on levels in CTL: MP supply, MP balance, the ratio of duodenal microbial protein (MCP) to MP supply (MCPMP), the stage of lactation (early or mid/late) and the type of forage (grass/legume- or corn silage-based). The MP supply and MP balance influenced negatively the relationship between  $\Delta$ MP and the response of true protein yield. Responses of milk urea, blood urea, and plasma urea cycle AA concentrations were associated positively with  $\Delta$ MP, indicating that a large amount of infused AA was catabolized to urea. Responses of plasma essential AA concentrations were related positively to  $\Delta$ MP. The relative effect of  $\Delta$ MP was highest for responses of plasma His concentration in cows fed grass/legume-based diets and at high MCPMP ratios. This relationship suggests that positive responses of plasma His concentrations are associated with diets relying heavily on microbial protein synthesis (high MCP), low in crude protein (low estimated MP supply), or both. The relationship between  $\Delta$ MP

and responses of plasma group 2 AA (Ile, Leu, Lys, and Val) concentrations was approximately 2 times greater than that for group 1 AA (His, Met, and Phe+Tyr) at mean MCPMP and MP supply. This could reflect the low hepatic removal group 2 AA compared with group 1 AA in dairy cows. Collectively, these results provide novel information on how dietary and cow conditions may alter responses to protein supplementation.

**Key words:** dairy cow, meta-analysis, casein, amino acid, protein yield

### INTRODUCTION

More than 2 decades ago, refined models were introduced to estimate protein supply and requirements for dairy cows. In these models, the supply of protein was no longer based on feed intake and the dietary concentration of CP (or ruminal degradability of CP), but rather on estimates of the flows of digestible protein and EAA. For example, the MP system from the NRC (2001) model and the protein digestible in the intestine system from Institut National de la Recherche Agronomique (INRA, 2007) proved to be major improvements over previous feeding systems. Both systems took into account the rumen degradability of CP and the contribution of microbial protein and RUP to MP supply. As a result, the net portal absorption of AA-N in ruminants is predicted more reliably and with less bias by MP supply estimations than by diet characteristics alone (Martineau et al., 2014).

Most models used to balance dairy rations use fixed efficiencies for the conversion of MP absorbed from the small intestine into MP required to support anabolic functions (Lapierre et al., 2014a). For example, one can compute that 1.5 kg of absorbed MP is required per kilogram milk true protein yield (MTPY), assuming a fixed efficiency of 0.67 as used in NRC (2001). This is despite the fact that research previous to NRC (2001) clearly indicated that the marginal recovery of

Received August 1, 2016.

Accepted May 17, 2017.

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absorbed AA into milk protein diminishes as the supply of duodenal protein increases (Whitelaw et al., 1986; Hanigan et al., 1998). Consequently, MTPY is overestimated at high MP supply and underestimated at low MP supply using fixed efficiencies. Not surprisingly, recent models are moving toward the implementation of variable efficiencies for the prediction of production responses to incremental MP or protein digestible in the intestine supply [e.g., NorFor (Volden et al., 2011) and new French feeding system (Sauvant et al., 2015)]. However, to be robust in the field, efficiencies need to be predicted with high accuracy. One way to increase the predictive accuracy for efficiencies is to study the response of MTPY to known increments of MP supply. Methodologically, more consistent production responses are expected in experiments where a known amount of MP is infused postruminally compared with experiments where differences in MP supply result from variations of dietary treatments (DePeters and Cant, 1992; Aikman et al., 2002; Chamberlain and Yeo, 2003).

Dose-response of MTPY to incremental MP supply can be achieved by postruminal casein infusion. Casein offers several advantages over other protein sources, as it is highly digestible and has an optimal AA profile for milk protein synthesis (Clark, 1975; Bionaz et al., 2012). Moreover, minimal effects of casein on rumen metabolism and feed intake are expected with postruminal infusion (Huhtanen and Hristov, 2010).

To our knowledge, the benefits associated with duodenal infusion of casein over oral administration were first demonstrated by Chalmers et al. (1954) in sheep, based on N balance and ruminal ammonia data. In dairy cows, Broderick et al. (1970) reported that abomasal infusion of sodium caseinate (800 g/d) decreased grain intake but increased milk protein yield and plasma concentrations of Ile, Leu, Phe, Val, and total EAA. Clark (1975) reviewed 7 studies and reported that postruminal casein infusion had the potential to increase milk yield (up to 4 kg/d) and milk protein yield (by 10 to 15%). These findings prompted more research with postruminal casein infusion under various experimental conditions.

Despite the large body of literature published since 1970, a comprehensive meta-analytic review is lacking on the relationships between postruminal casein infusion and variables on production and concentrations of plasma AA and blood urea in dairy cows. Some issues with previously published reviews include (1) part of the literature was reviewed at time of publishing (e.g., Hanigan et al., 1998; Patton et al., 2015); (2) experiments with postruminal casein infusion and AA infused i.v. were aggregated (e.g., Doepel et al., 2004; Lapierre et al., 2012); or (3) only the relationship between casein infusion and milk protein yield was evaluated (e.g.,

Huhtanen and Hristov, 2010). Recently, Patton et al. (2015) reported the relationships between casein infusion and production variables and plasma EAA concentrations, but included no mention of the influence of other factors (e.g., casein infusion rate).

The relationship between casein infusion rate (as a primary variable of interest) and the response of DMI was reported in a previous paper (Martineau et al., 2016). Martineau et al. (2016) indicated that for outcomes other than the response of DMI, the primary variable of interest should be the difference in estimated MP supply ( $\Delta$ MP) between casein-infused and control treatments due to within-study differences in DMI and also to ration composition, in rare occurrences (e.g., Rogers et al., 1984).

Our hypothesis was that summarizing results from several casein infusion experiments would allow for determination of the response of several variables to incremental MP supply and the possible influence of explanatory variables on these relationships. For example, the supply and balance in MP, the ratio of microbial CP to MP supply (**MCPMP**), the stage of lactation (early or mid/late), and the type of forage (grass/legume- or corn silage-based diets) might influence the relationships between  $\Delta$ MP and responses of MTPY and plasma EAA concentrations. Therefore, the objectives of the current meta-analysis were to (1) review the relationships between  $\Delta$ MP and the responses of production variables and plasma AA and blood urea concentrations in lactating dairy cows; (2) consider  $\Delta$ MP as the primary variable of interest in all models; and (3) explore the influence of other explanatory variables on the relationship between  $\Delta$ MP and each response.

## MATERIALS AND METHODS

### Database

The Scopus online database was searched in November 2015 for studies where casein was infused postruminally in lactating dairy cows with the terms “casein or caseinate,” “abomasal or duodenal,” and “cow or dairy.” Inclusion criteria were (1) a treatment consisting of intact casein infused postruminally (**CAS**); (2) a control treatment (**CTL**) consisting of water or saline infused postruminally; (3) number of experimental units (**n**) for CAS and CTL; (4) a measure that allows calculation of the variance of the mean difference (CAS minus CTL); (5) information on feed intake, feed ingredients, or ration composition; (6) estimated MP supply in CTL >800 g/d; and (7) published in English or French.

The infusion of a substrate in a single treatment was an exclusion criterion unless it was infused in both CAS

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