J. Dairy Sci. 98:1–18
http://dx.doi.org/10.3168/jds.2014-8496

Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows

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ABSTRACT

This study investigated the effect of metabolizable protein (MP) supply and rumen-protected (RP) Lys and Met supplementation on productivity, nutrient digestibility, urinary N losses, apparent total-tract digestibility of dietary AA, and the efficiency of AA utilization for milk protein synthesis in dairy cows. The experiment was conducted with 8 ruminally cannulated Holstein cows in a replicated 4 × 4 Latin square design trial with 21-d periods. Treatments were (1) MP-adequate diet (AMP; MP balance of −24 g/d); (2) MP-deficient diet (DMP; MP balance of −281 g/d); (3) DMP supplemented with 100 g of RPLys/cow per day (estimated digestible Lys supply = 24 g/d; DMPL; MP balance of −305 g/d); and (4) DMPL supplemented with 24 g of RPMet/cow per day (estimated digestible Met supply = 15 g/d; DMPLM; MP balance of −256 g/d). Diet had no effect on total-tract nutrient digestibility, milk production, and milk composition, but the DMP diets decreased urinary N excretion and the ammonia emitting potential of manure. Plasma Met concentration was increased by DMPLM compared with AMP. Supplementation with RPLys had no effect on plasma Lys. Concentration of most AA in milk protein was increased or tended to be increased by DMPLM compared with DMPL. Except for the AA supplemented as RPAA (i.e., Met and Lys), apparent total-tract digestibility of all dietary AA was generally greater for the DMP diets and ranged from 33% (Arg, AMP diet) to 67% (Thr, DMPL diet). Apparent recovery of dietary AA in milk protein followed the same trends, being greater for the DMP diets than AMP and generally lower for Lys and Met with the RPAA-supplemented diets versus AMP and DMP. The RPAA were apparently not used for milk protein synthesis in the conditions of this experiment. The AA recoveries in milk protein varied from around 17% (Ala) to 70% (Pro). Milk protein recoveries of essential AA (EAA) were around 54% for the DMP diet and 49% for AMP. The estimated efficiency of utilization of digestible EAA for milk protein synthesis was generally greater for the DMP diets compared with AMP. In this trial, blood plasma Lys and Met were labeled by abomasal pulse-dose of ^{15}N-Lys and ^{13}C-Met (respectively). Analysis of the ^{15}N-Lys and ^{13}C-Met decay curves in plasma indicated trends for a faster extraction of Lys and Met from plasma for the MP-deficient diets, compared with AMP. Overall, this study confirmed conclusions from previous analyses that the efficiency of utilization of dietary EAA will increase with decreasing MP-AA supply.

Key words: dietary protein, rumen-protected methionine, rumen-protected lysine, amino acid utilization, dairy cow

INTRODUCTION

Protein nutrition of the dairy cow is 2-dimensional with dietary proteins providing N for microbial protein synthesis in the rumen and then complementing microbial protein to supply AA for maintenance, growth, reproduction, and milk protein synthesis by the cow. Although the rumen (and the ruminant animal) can function on NPN alone (Virtanen, 1966), adequate supply of essential and limiting AA is critical for maintaining the high milk production of modern dairy cows (NRC, 2001). Production responses in earlier studies and AA concentrations in plasma, rumen microbial, milk, and common feedstuff proteins have identified Met, Lys, and His as most limiting AA in dairy cows in various intensive production systems (Griel et al., 1968; Broderick et al., 1974; Vanhatalo et al., 1999; Schwab et al., 2005). Dietary supplementation of rumen-protected (**RP**) forms of Met and Lys has been extensively studied, but production responses have not been consistent (Patton, 2010; Robinson, 2010), perhaps partially due to oversupply of MP and AA in some of the studies used in these meta-analyses.

Several analyses concluded that the transfer efficiency of MP and individual AA into milk protein is not

Received June 17, 2014.
Accepted November 17, 2014.
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constant and decreases with increasing supply (Doepel et al., 2004; Metcalf et al., 2008). Similar trends have been reported for supplemental RPMet and RPLys by Vyas and Erdman (2009). In that study, the authors estimated sharp decreases in the marginal efficiency of Met and Lys utilization for milk protein synthesis with increasing AA supply. A recent meta-analysis by Martineau et al. (2014) concluded that the efficiency of transfer of feed N into AA-N appearing in portal circulation is increased when dietary protein is in short supply. An analysis of a data set from trials in which cows were fed at or below MP requirements showed apparent efficiency of utilization of predicted (NRC, 2001) digestible Met, Lys, and His supplied at the duodenum for synthesis of milk AA of 72 ± 0.72, 61 ± 0.65, and 61 ± 0.68%, respectively, once requirements for maintenance are removed from AA supply (A. N. Hristov, unpublished data). The conversion coefficients, however, varied largely among individual cows and were negatively correlated with MP supply ($r = -0.37$ to -0.46). Although the efficiencies calculated using this approach are likely overestimated due in part to overestimation of maintenance AA requirements by NRC (2001), particularly metabolic fecal protein (**MFP**; Ouellet et al., 2010; Arriola Apelo et al., 2014), they are reflective of the negative relationship between efficiency of utilization and supply of MP or individual AA. In the meta-analysis of Doepel et al. (2004), the efficiency of utilization of available Lys and Met for milk AA synthesis was 90% at 50% of optimum AA supply and decreased to 60% at 125% of optimum supply. Thus, it is apparent that the efficiency of utilization of MP-AA for milk protein synthesis in dairy cows is not constant (as assumed by NRC, 2001), but depends on supply and demand. Apart from the modeling data mentioned above, experimental evidence in support of this concept was observed by Raggio et al. (2004) and Lemosquet et al. (2010), where the nonproductive removal of AA across tissues increased with AA supply.

Therefore, the main objective of this experiment was to estimate apparent efficiencies of utilization of dietary AA for milk protein synthesis in dairy cows fed MP-adequate or MP-deficient diets, according to NRC (2001). Our hypothesis was that increased AA supply would decrease the apparent efficiencies of use of dietary and digestible AA for milk protein synthesis. A secondary objective was to evaluate the production effects and estimate the utilization efficiency of use for milk protein synthesis of RPMet and RPLys supplemented to an MP-deficient diet.

MATERIALS AND METHODS

Animals involved in these experiments were cared for according to the guidelines of the Pennsylvania State University Animal Care and Use Committee. The committee reviewed and approved the experiment and all procedures carried out in the study.

Animals and Experimental Design

This experiment used 8 multiparous (2.8, SD = 0.7 lactations) Holstein dairy cows in a replicated 4 × 4 Latin square design, balanced for residual effects, with 2 concurrent squares and four 21-d periods. At the beginning of the trial, BW of the cows averaged 684 kg (SD = 68), DIM, 102 d (SD = 28), and milk yield, 43 kg/d (SD = 5.3). For the first 12 d of each experimental period, cows were housed in the tie-stall facility of Pennsylvania State University's Dairy Center. During the last 9 d of each period, cows were moved to metabolic stalls, in which total fecal and urine collections were conducted. All cows were fitted with 10-cm ruminal cannulas (Bar Diamond Inc., Parma, ID) and randomly assigned to one of the following treatments: (1) MP-adequate diet (**AMP**; MP balance of −24 g/d); (2) MP-deficient diet (**DMP**; MP balance of −281 g/d); (3) DMP supplemented with 100 g of RPLys/cow per day (**DMPL**; MP balance of −305 g/d); and (4) DMPL supplemented with 24 g of RPMet/cow per day (**DMPLM**; MP balance of −256 g/d). The MP balance was estimated based on NRC (2001) requirements at treatment average DM intake, milk yield, and milk composition measured during the trial and nutrient composition of the diets (Table 1). AminoShure-L (Balchem Corporation, New Hampton, NY) and Mepron (Evonik Industries AG, Hanau, Germany) were used as sources of RPLys and RPMet, respectively. The application rates of RPLys (100 g/animal per day) and RPMet (24 g/animal per day) were designed to meet the requirements of digestible Lys (**dLys**) and Met (**dMet**) of the cows, assumed at 6.6 and 2.2% of the MP requirements, respectively (Schwab et al., 2005). The supply of dLys and dMet from AminoShure-L and Mepron were estimated at 24 and 15 g/cow per day using the manufacturers' data (AminoShure-L, 38% Lys and 64% bioavailability; and Mepron, 85% Met and 72% bioavailability). Diets were fed as TMR once daily at 0800 h targeting 5 to 10% refusals. The RPLys and RPMet were top-dressed, mixed with a small portion of the freshly delivered TMR.

Sampling and Measurements

During the entire experiment, TMR offered and refusals were recorded daily. Samples of TMR, refusal, and individual feed ingredients were collected twice weekly. Samples were dried for 48 h at 65°C in a forced-air oven and ground in a Wiley Mill (A. H. Thomas Co.,