# Variation in Lysine Supply…
# When Does it Happen and When Does it Matter?

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## INTRODUCTION

Lysine (Lys) is a diamino acid (α- and ε-amino groups) with a basic side chain that occurs naturally as an L-isomer. Lysine can be synthesized by both plants and bacteria from aspartate through the diaminopimelate pathway (Azevedo et al., 2006; Fan et al., 2010) and by fungi from α-ketoglutarate thorough the α-aminoadipate pathway (Nishida and Nishiyama, 2000); however, these pathways are absent in mammals. Additionally, mammals do not possess a transaminase that can convert the α-keto analogue of Lys, α-keto-ε-aminocaproic acid, into L-Lys which eliminates the alternative of supplementing diets with D-Lys (Friedman and Levin, 2012) or α-keto-ε-aminocaproic acid (Baker, 1986). Consequently, Lys is nutritionally classified as an essential amino acid.

Lysine's main function is as a substrate for protein synthesis. In chromosomes, Lys is found in high concentration in histones (Kinkade, 1969). Histones are proteins that associate with DNA to form nucleosomes, main subunits of chromatin, and aid in the compaction of DNA (Wolffe, 1991). Modifications such as methylation or acetylation of the Lys residues in histones result in the activation or repression of the transcription of genes (Martin and Zhang, 2005). In mammalian tissue, Lys is found in major proteins such as collagen and elastin (Reiser et al., 1992). Pathways that involve the formation of aldehydes from Lys or hydroxylysine residues in collagen and from Lys residues in elastin are crucial in the formation of cross-links that give these proteins their structural properties (Eyre et al., 1984). Another important function of Lys is to serve as the carbon backbone for carnitine synthesis (Vaz and Wanders, 2002). Inside the cell, the carnitine shuttle system permits long-chain fatty acids to gain access from the cytosol into the mitochondrial matrix where β-oxidation occurs and energy is generated. Carnitine is also involved in peroxisomal fatty acid oxidation, branched-chain amino acid (AA) oxidation, and the removal of acyl groups in urine (Hoppel, 2003).

In dairy nutrition, Lys is an important nutrient and it represents 16.0% of the total essential AA contained in milk (NRC, 2001). Lysine is generally identified as the first-limiting AA in diets fed to lactating dairy cows that contain high inclusion of corn or corn co-products such as distillers dried grains with solubles (DG; Schingoethe et al., 2009). The rise of the grain-ethanol industry has resulted in a dramatic increase in the availability of DG. Furthermore, because the feed is low in Lys, many studies used to examine the effect of this feed in dairy rations also serve as good examples of what may happen when the supply of Lys is altered. In general, this feed ingredient is characterized by a high concentration of CP (30% of DM) and energy (2.26 Mcal/kg of $NE_L$; Schingoethe et al., 2009), but low concentration of Lys (1.86% of RUP; Kelzer et al., 2010). Furthermore, heat applied during production of DG can reduce the bioavailability of Lys through Maillard reactions. Despite low levels of metabolizable Lys coming from DG, only a few studies (Owen and Larson, 1991; Kleinschmit et al., 2006; Mjoun et al., 2010b) have reported negative impacts on milk protein when feeding DG. Conversely, positive impacts on milk protein when feeding DG have been observed (Mjoun et al., 2010a). As the cost of feeds that have traditionally been used for energy and protein continues to increase, there is a need to understand the potential impact on milk yield and composition of replacing these feeds with DG. Additionally, given that DG are low in Lys, the many feeding studies published examining this feed shed valuable insight into practical conditions where the supply of this AA varies and then demonstrate the impact on milk protein production.

## IMPACT OF LYSINE SUPPLY ON MILK PROTEIN, AN EXAMPLE WITH META-ANALYTIC EVALUATION OF DISTILLERS GRAIN

Recently we have conducted a meta-analysis to evaluate the inclusion of corn DG on milk protein production and to evaluate the relationships involving the inclusion of corn DG, the supply of Lys and methionine (Met), and milk protein (Paz et al., 2013). To do so, a database was built upon peer-reviewed articles published after 2001 that were related to dairy feeding experiments which included diets that contained at least one of the following corn milling co-products: dried DG, high protein DG (HPDG), low-fat DG (LFDG), and wet DG. Selection of publications after 2001 was set to capture articles that were not used to develop the latest version of the National Research Council model (NRC, 2001). From each article, data of the reported animal description, milk production and composition, ingredient composition of the diets, and chemical composition of the ingredients were entered into the NRC model (2001) to predict $NE_L$- and MP-allowable milk production, Lys and Met flows, and concentration of Lys and Met in MP (MP-Lys and MP-Met, respectively). Values reported by the NRC (2001) feed tables for the chemical and AA composition of forages and corn milling co-products were used when these data were not reported.

Within study, response of milk protein was calculated as the difference between cows fed the DG diet and those fed control diet (Hollmann et al., 2011). A positive response value represented a positive response in milk protein while a negative value value did not. A subset of data was used to determine the relationships between lactational response variables and the predicted Lys and Met flows and between the lactational response variables and MP-Lys and MP-Met. Data were restricted to diets where MP-allowable milk was predicted to be lower than $NE_L$-allowable milk and where MP balance was between -350 and +60 g/d. The latter restrictions aimed to select diets where MP was more limiting than $NE_L$ and where Lys and Met were limiting (Schwab, 2004). As expected, increasing the inclusion of DG reduced the flow of Lys (Figure 1).

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Overall, the concentration of milk protein was not associated with the inclusion of DG. In contrast to DG, an increase in Lys and Met flows had a significant and positive effect on the concentration of milk protein (Figure 2). This was expected as it is widely known that the changes in the flow of these AA are important for milk protein synthesis (Schwab et al., 1976). These observations are in agreement with the data summarized in the Dairy NRC (2001) which reports that milk protein concentration is responsive to postruminal supplies of Lys and Met. It is important to note that all of the studies in the current analysis were published subsequent to 2001, and, as such, none of them were used in database for creating the NRC (2001) model. To illustrate the impact of the inclusion of DG on the flow of Lys and Met, we sorted the data to create a subset that included only studies that fed diets with increasing concentrations of corn DG, and, as expected, as dietary concentration of DG increased the flow of Met was not affected (Figure 1) but the flow of Lys (Figure 1) was

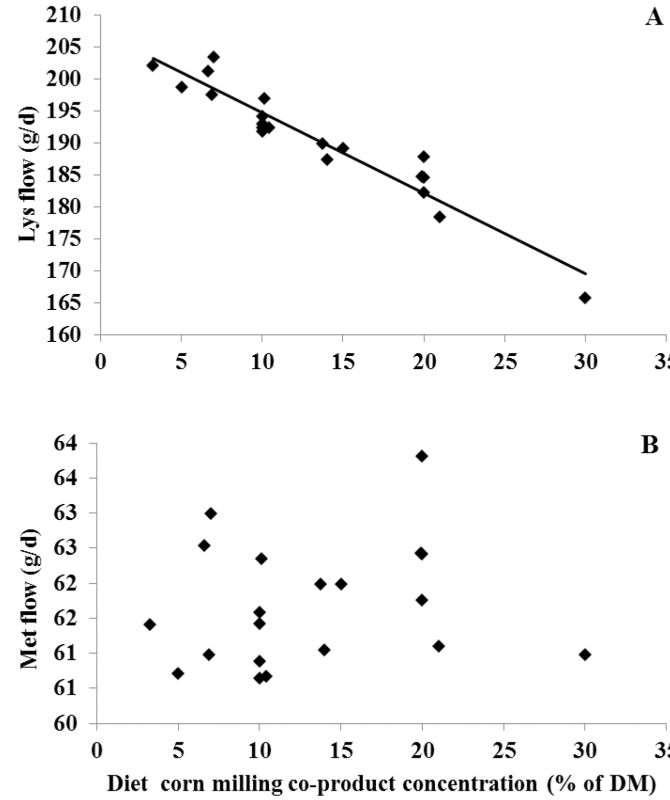


**Figure 1.** Relationship between (A) Lys flow to the small intestine (solid line is y = -1.2596x + 207.28; *P* = < 0.01; $R^2$ = 0.91) and (B) Met flow to the small intestine (*P* = 0.79) and dietary corn milling co-product concentration (% of DM).

reduced. However, although the inclusion of DG resulted in a reduced flow of Lys, it is possible that the supply of Lys was adequate to maintain milk protein. Practically, we believe that this is the case when diets are high in CP (i.e. > 18%) and despite having a low concentration of Lys, no negative effects on milk protein are observed. This is because the total flow of Lys still meets the need of the mammary gland. Thus to determine if diets containing DG could be limiting in Lys, we sorted the data to create an additional subset where the calculated requirement of energy was met, but MP was predicted to be either limiting or close to being limiting (-350 and +60 g/d; Schwab, 2004). We then evaluated the relationship between the flow of Lys and Met and the impact on milk protein. We expressed the flow of Lys and Met in two ways: first, as the percent of each AA in the total MP and second, the predicted flow (g/d) to the duodenum. In doing so, we were interested in evaluating the relationship between the predicted supply of these AA and the response in milk protein. When the concentration of AA was regressed against the response in milk protein, a positive trend was observed between response in the concentration of milk protein with increasing the concentration of MP-Lys (Figure 2). The observed response in increasing concentrations of MP-Lys suggests that when feeding DG, cases may exist where animals are deficient in Lys but that this usually occurs when the supply of MP is very close to requirements. This would commonly be the case in diets formulated to contain low concentrations of CP and also when cows are producing high amounts of milk.

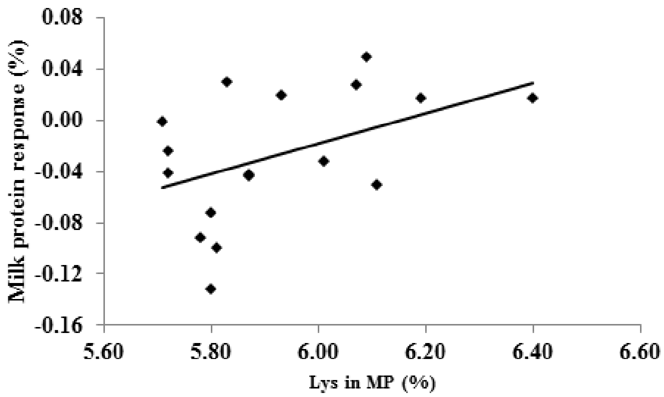


**Figure 2.** Relationship between milk protein response (corn distillers grains and solubles diet minus control diet) and Lys in MP (solid line is y = 0.118x - 0.7212; *P* = 0.09; $R^2$ = 0.24).

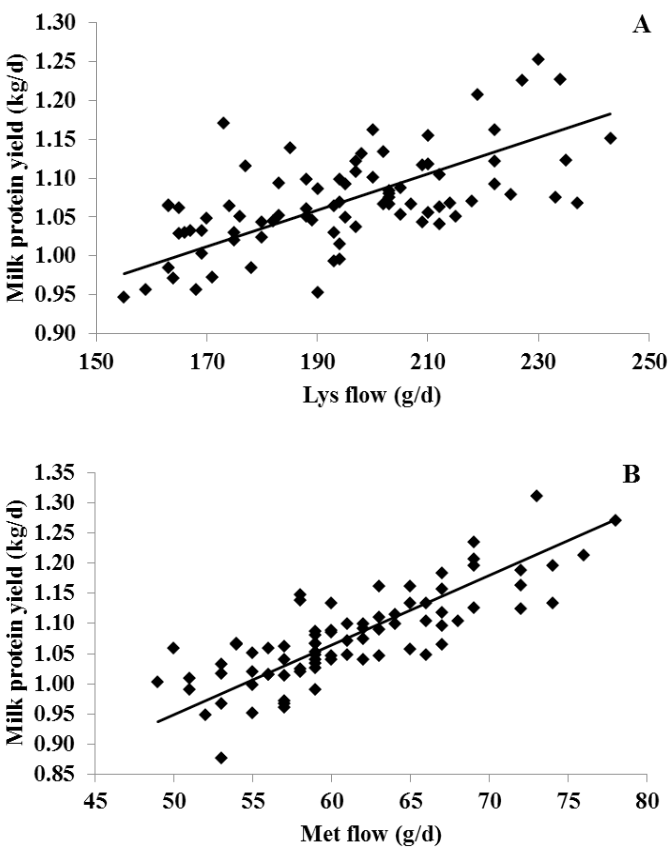


**Figure 3.** Relationship between milk protein yield and (A) Lys flow (solid line is y = 0.00232x + 0.6169; *P* = < 0.01; $R^2$ = 0.34) and (B) Met flow (solid line is y = 0.01143x + 0.3733; *P* = < 0.01; $R^2$ = 0.61).

The NRC (2001) publication has proven to be a powerful and accurate tool for dairy nutritionists to evaluate the predicted supply of AA in a given ration. More specifically this publication notes that the optimal level of Lys and Met in MP is estimated to be 7.2% and 2.4%, resulting in the 3.0:1.0 ratio. However because these levels are difficult to achieve in most practical settings, the committee suggested 6.6% and 2.2% as practical targets of Lys and Met respectively. Unfortunately, data for this analysis did not contain any observations beyond this range, thus we could not fully test the relationships as outlined by the NRC (2001). We were however able to test the relationship between the predicted supply of Lys and Met on total production of milk protein. The relationship between these AA and milk protein yield was positive (Figure 3), and the strong relationships observed in these figures suggest that when assessing AA supply in dairy rations it may be more insightful to evaluate the predicted total supply (g/d) than to simply evaluate the concentration in MP. Furthermore, these results also illustrate the impact of total supply of AA on milk protein. Practically, ration evaluation procedures, which simply evaluate the concentration of AA in MP, may be misleading if concentrations of Lys and Met are low but the amount of CP is high enough to deliver adequate amounts of these AA.

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