Multifaceted Roles of Lysine in Dairy Cattle

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Introduction

Lysine (Lys) and methionine (Met) are consistently identified as the first-limiting essential amino acids in dairy cow diets. Both are critical for milk protein synthesis and play emerging roles in regulating immune and antioxidant function. While these amino acids (AA) are traditionally recognized as a limiting amino acid for milk protein synthesis in dairy cows, there is an expanding body of evidence highlighting their broader role in regulating hepatic metabolism, mammary gland metabolism, antioxidant defense, and immune function.

A recent meta-analysis of 12 studies with 40 treatments confirmed that increasing Lys supply improves milk and milk protein production, with responses influenced by stage of lactation and duration of feeding Lys in the postpartum period (Arshad et al., 2024a). Beyond its role as a substrate for protein synthesis, Lys contributes to metabolic regulation through carnitine production and interactions with other amino acids, particularly methionine. Precision supplementation with rumen-protected Lysine (RPL) has therefore emerged as a practical tool to optimize nutrient efficiency, reduce reliance on variable protein sources, and support both production and environmental goals in modern dairy systems.

Previously, studies in bovine hepatocytes demonstrate that different forms of Met and its analogs, such as 2-hydroxy-4-(methylthio)butanoic acid (HMB), modulate genes controlling transmethylation, transsulfuration, and regeneration pathways, with consistent reductions in methionine cycle activity as Met supply increases (Zhang et al., 2016; Zhang and White, 2017, 2019) . Beyond its metabolic functions, Met-derived compounds such as S-adenosylmethionine and glutathione are central to redox balance and the inflammatory response, linking dietary supply to immunometabolic resilience. These mechanistic insights provide a foundation for understanding recent in vivo findings on how Met supplementation influences hepatic bioenergetics and production responses during the transition period in dairy cows. Interestingly, these prior in vitro studies revealed metabolic support for the Met:Lys ratio commonly referenced in the field when it was identified that the ratio of Met:Lys, not just the amount of Met, was important to mitigate the inflammatory response to LPS challenge in vitro (Zhang and White, 2017).

Replacing Porcine Blood Meal with RP Lys

Given the importance of ensuring that Lys and Met needs are met within dairy cattle diets, rumen-protected (RP) amino acids offer a practical alternative, allowing nutritionists to target limiting AA directly; however, the extent to which a complex

metabolizable protein source (e.g. porcine blood meal, PBM) can be replaced with only the most limiting, or two most limiting AA, is not yet fully elucidated. In a recent study, we investigated (1) the effects of partially or fully replacing PBM with RPL on production and nutrient partitioning (Arshad et al., 2025a) and (2) the effects of MP adequacy on $CD4^+$ T lymphocyte bioenergetics (Arshad et al., 2025b). Traditionally used as a source of AA, PBM has a valuable AA profile but its use is increasingly limited due to variability, cost, and regulatory restrictions. In this study, we replaced increasing amounts of PBM with RPL, while maintaining metabolizable Met, without supplementing other AA. Sixty-four mid-lactation Holstein cows were assigned to one of four diets for 12 weeks: PBM (control), or diets where 33% (RPL33), 66% (RPL66), or 100% (RPL100) of PBM-derived Lys was replaced with RPL. Methionine supply was maintained across treatments with RP-Met. Data collected included dry matter intake (DMI), milk yield and composition, nitrogen (N) balance, gas exchange, and blood metabolites. Nutrient partitioning was assessed via respiratory quotient, carbohydrate and fat oxidation, and heat production.

Replacing Lys from PBM with RPL did not alter body weight, body condition score, or estimated body energy balance, suggesting that overall energy status was maintained regardless of the source of Lys, despite a linear tendency for reduced (P = .06) DMI with increasing RPL replacement. Milk yield was similar for cows fed the PBM, RPL33, and RPL66 treatments although it was significantly reduced in the cows fed the RPL100 diet (49.6 kg/d vs. 45.9 kg/d, PBM vs. RPL100, respectively). Milk fat percentage increased linearly (P = 0.02) with RPL inclusion, whereas protein percentage tended to be reduced (P = 0.09) linearly. Overall, the total milk solids increased linearly (P = 0.04) and energy-corrected milk yield only tended to be reduced linearly (P = 0.08).

Nitrogen intake decreased linearly with RPL inclusion due to lower dietary crude protein content. Milk nitrogen output also declined, but fecal and urinary nitrogen excretion were not affected. Consequently, nitrogen balance and nitrogen use efficiency (milk N as a proportion of intake N) were similar across treatments, although cows on higher RPL diets partitioned less nitrogen toward milk protein. Milk urea nitrogen concentrations decreased with RPL, consistent with reduced nitrogen intake.

Gas exchange measurements showed that enteric methane and carbon dioxide emissions were not influenced by diet; however, oxygen consumption was reduced at RPL66 and RPL100, suggesting a decrease in oxidative metabolism. Methane yield (g/kg DMI) and intensity (g/kg ECM) remained similar across treatments. Blood metabolite concentrations, including glucose, fatty acids, β-hydroxybutyrate, and liver enzyme activities, were not altered by treatment. Blood urea nitrogen declined slightly with RPL inclusion, again consistent with reduced nitrogen intake.

In order to investigate the impact of metabolizable protein on immune function, cows (n=32) from the two extreme treatment groups were used for additional analysis. Blood was collected from cows fed the PBM diet (MP-adequate; 3,111 g/d MP) or 100% RPL diet (MP-deficient; 2,983 g/d MP) to isolate $CD4^+$ T lymphocytes at week 11 and

analyzed for mitochondrial and glycolytic function using a Seahorse extracellular flux analyzer. Cells were studied under resting (non-activated) and stimulated (activated) conditions. Cows fed the MP-deficient diet had reduced maximal respiration and spare respiratory capacity in both resting and activated $CD4^+$ T cells. Glycolytic activity (extracellular acidification rate) declined progressively over time in MP-deficient cows. Mitochondrial and glycolytic ATP production rates were unchanged, but immune persistence appeared compromised.

These studies highlight the dual importance of amino acid nutrition for both production and immune resilience. While RPL can effectively substitute PBM to meet up to 66% of the Lys requirement, complete replacement reduces total MP supply and impairs milk yield. This demonstrates the need to preserve MP adequacy while balancing limiting AAs. Additionally, modest MP deficiency reduced the metabolic fitness of $CD4^+$ T lymphocytes, which may compromise immune responses. This contributes to growing evidence that Lys, Met, and their relative availability, play an important role in immune function.

Conclusions

Maintaining adequate MP supply while balancing Lys and Met is essential for optimizing production, nutrient efficiency, and immune competence. This research indicates that partially replacing PBM with RPL, while maintaining metabolizable Met but allowing a modest reduction of MP, maintained overall production, ECM to feed rations, and nitrogen use efficiency. In contrast, full replacement of PBM with RPL resulted in reduced SMI, milk yield, milk protein yield, and a tendency for reduced ECM. Fully replacing PBM with RPL also linearly decreased nitrogen intake and manure nitrogen excretion without influencing overall nitrogen balance. Take together, these results suggest that partial substitution of PBM with RPL is practical as long as total metabolizable protein supply is maintained. Precision nutrition strategies should ensure MP adequacy, while fine-tuning Lys and Met balance, especially during periods of metabolic or immune stress.

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