METABOLISM AND PERFORMANCE RESPONSES TO PROTEIN AND AMINO ACID SUPPLY – LATEST APPLICATIONS

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INTRODUCTION

Increased costs of dietary protein and scrutiny of dairy's environmental impact has renewed interest in altering the supply of protein provided to lactating cows. Reductions in dietary crude protein and supplying the optimal ratio of amino acids (AA) has been a successful strategy to increase efficiency of nitrogen utilization for milk and milk protein synthesis in dairy cows. Continued research has shown that specific AA, lysine (Lys) and methionine (Met), have a role that extends beyond increasing protein production in lactating dairy cows. The objective of this paper are to describe protein metabolism, physiological responses to altered protein and AA supply, as well as highlight new roles for AA in the dairy industry.

INEFFICIENCY IN NITROGEN CONVERSION TO MILK PROTEIN

Increasing efficiency of nutrient utilization for conversion into milk is important for the sustainability of the dairy industry. Efficiency can be defined as conversion of feed nutrients into milk and milk components. Conversion of dietary N into milk protein by dairy cows is a limitation ranging from 12 to 36% of dietary N being converted into milk and 64 to 88% of ingested N potentially released through volatilization or leaching into the environment (Moorby and Theobald, 1999; Castillo et al., 2000; Spears et al., 2003). This nutrient loss also represents a significant economic loss for dairy farmers, making protein one of the most expensive nutrients for dairy cows (VandeHaar and St-Pierre, 2006). Moreover, increasing transfer of dietary protein into milk protein is not as simple as increasing dietary protein content to increase N output in milk as this rarely improves efficiency of N utilization above 25 to 30% (Kohn et al., 1997; St-Pierre and Thraen, 1999; Ipharraguerre and Clark, 2005). This suggests the inability to increase N efficiency above 30% may be linked to inefficiency in tissue specific utilization of amino acid N (AA-N).

PROTEIN METABOLISM

Metabolizable protein (MP) requirements of ruminants are met through the combination of rumen microbial protein and dietary protein escaping rumen degradation. Rate and extent of ruminal protein degradation determine nitrogen (N) availability to microorganisms in the rumen as well as supply of amino acids (AA) to small intestine of the cow. The fraction of consumed protein subject to proteolysis by rumen microbes, rumen degradable protein (RDP), results in peptides and AA which are transported into microbial cells for further degradation, incorporation into microbial protein, or deamination to volatile fatty acids, resulting in CO_2, and ammonia

(Tamminga, 1979). These peptides and AA, along with carbohydrates serve as an energy and N sources for rumen microbial populations. Microbial synthesis relies on adequate supply and type of carbohydrates for synthesis of peptide bonds (Stern and Hoover, 1979) and alteration in carbohydrates can result in improved N uptake by rumen microbes. The products of ruminal protein degradation, microbial protein, constituents of microbial and bacterial origin, ammonia, and endogenous N sources combine with the protein fraction escaping ruminal proteolysis, rumen undegraded protein (RUP), and flow to the small intestine for further proteolytic action and absorption (Armstrong, et al., 1977). Flow of microbial and dietary protein to the small intestine dictates protein quantity and AA profile available for absorption from the small intestine. The magnitude of microbial contribution to MP has a similar effect. Intestinal proteolysis further alters AA profile available for tissue use in protein synthesis and turnover (NRC, 2001). Optimizing efficiency of protein utilization in ruminants is dependent on supplying adequate quantities of substrates for microbial growth and meeting the total AA needs of tissues for production of milk, meat, or wool per unit of nutrient input to be achieved (NRC, 2001).

Metabolizable protein must match tissue needs for AA in order to maximize production. Failure to supply adequate AA for tissue utilization often arises when the contribution of AA from dietary protein sources and microbial protein fail to provide the necessary profile of AA for milk protein synthesis. Inability to supply adequate AA from RUP is further exacerbated by use of corn and distiller's co-products which are typically low in Lys (Nichols et al., 1998; Schingoethe, 1996). Improving quantification and prediction of post-absorptive AA metabolism, especially Lys and Met AA necessary for milk protein synthesis, has been identified as a critical need to improve feeding and management practices aimed at meeting protein and AA requirements (Hanigan et al., 2000). Furthermore, indicators of AA turnover and exchange between tissues are a critical aspect in the understanding of AA metabolism and whole body utilization (Lapierre and Lobley, 2001; Lobley and Lapierre, 2003).

MANIPULATING PROTEIN METABOLISM

Altering milk protein yield via nutritional manipulation has proven to be challenging (DePeters and Cant, 1992). Increasing RUP at the expense of RDP may compromise the quality of MP and supply of AA to tissues, including mammary tissue for milk protein synthesis (Santos et al., 1998). Coincidently, post-ruminal protein infusions have shown consistent increases in milk protein yields (Tyrell et al., 1972; Derrig et al., 1974; Guinard et al., 1994). The discrepancy between manipulations of MP by diet or post-ruminal infusion suggests that the current state of understanding ruminant AA metabolism does not accurately predict milk protein yields (Doepel et al., 2004).

Net protein absorption represents the transfer of AA and peptides into hepatic portal blood and is subject to hepatic extraction and metabolism. Predicting changes in the rate and extent of AA metabolism by liver is an essential prerequisite for improved precision of models that predict MP requirements for dairy cows (Hanigan et al., 2004a). Post-ruminal casein infusion consistently increases milk and milk protein yield,

supporting that protein supplied by casein provides postruminal AA that are absorbed for protein synthesis (Clark et al., 1977; Lemosquet et al., 2009; Galindo et al., 2011). Post-ruminal casein infusion of 600 g/d or greater increases milk protein yield by 11 to 19% and milk yield by 7 to 11% (Guinard et al., 1994; Galindo et al., 2011) without subsequent increases in dry matter intake. This variability suggests that responsiveness of protein yield to post-ruminal protein is a function not only of AA supply (Hanigan et al., 1998) but also the efficiency of post-absorptive AA metabolism, a combined function of hepatic AA catabolism, mammary AA extraction and conversion to milk protein (Lapierre and Lobley, 2001; Lobley and Lapierre, 2003).

In a recent study (Tucker et al., 2013) post-ruminal infusion of protein increased ($P \leq 0.05$) milk yield by 10.5%, milk protein yield by 20%, milk urea N by 23.5%, blood urea N by 18.6%, and abundance of ornithine transcarbamoylase mRNA by 52.8% (Table 1). However when other indices of nitrogen catabolism, as well as Lys and Met catabolism were measured, post-ruminal infusion of protein did not alter ($P > 0.10$) abundance of argininosuccinate synthase, aminoadipate semialdehyde synthase, cysteine sulfinic acid decarboxylase, and cystathionase mRNA as well as altered metabolism of particular AA. These data indicate increased ureagenesis matched by up-regulation of non-essential AA catabolism and a disproportional increase in Lys oxidation in response to increased post-ruminal protein infusion. Greater circulating concentrations of urea N have been shown to indicate increased AA catabolism potentially due to greater amounts of AA utilized for milk protein synthesis, use by alternate tissues, or alternate metabolic fate (Larsen et al., 2014). Combined these data suggest differential AA metabolism in liver which ultimately regulates the profile of circulating AA available for milk protein synthesis.

Table 1. Least squares means for lactational performance, blood metabolite, and hepatic rates of degradation of early lactation dairy cows supplied increased metabolizable protein through abomasal infusion.

	Milk Protein Isolate Infusion		SE	*P* - value
	0 g/d	600 g/d		
DMI, kg	15.2	15.7	0.3	0.12
Milk Yield, kg	28.2	31.2	1.1	< 0.05
Milk Fat, g	885	1030	85	< 0.05
Milk Protein, g	649	781	30	< 0.05
Milk urea N, mg/dL	10.4	13.6	0.8	< 0.05
Plasma Urea N, mg/dL	13.4	15.9	0.6	< 0.05
Rate of Degradation, nmol product/mg tissue/h				
L-[U ^{14}C] Lysine	0.063	0.143	0.04	0.05
L-[1-^{14}C] Methionine	0.025	0.027	0.005	0.71
L-[methyl-^{14}C] Methionine	0.020	0.025	0.01	0.72
L-[U ^{14}C] Alanine	0.37	0.52	0.06	0.07

AMINO ACIDS AND THE LACTATING DAIRY COW

Tissue needs for AA are determined by the balance of AA for protein synthesis and AA that undergo catabolism (Lapierre et al., 2012). In ruminants protein for tissue metabolism is a combination of ruminal protein synthesis and dietary protein that escapes rumen degradation. Amino acid catabolism, and therefore availability, for tissue and milk synthesis is a function of energy intake, rate of growth and production, maintenance needs, health, and activity levels. Growth and production are suppressed when AA supply is compromised or fails to match the profile of AA needed. Likewise, the composition of growth and milk can be altered by inadequate AA supply (NRC, 2001). Inadequate supply of AA, resulting in decreased milk synthesis or altered milk composition compromises farm profits through decreased product formation and increased N losses to the environment. Biomarkers of AA adequacy and efficient utilization are sought to improve precision of diet formulation for lactating dairy cows that would result in decreased N excretion and improved production.

Research utilizing AA extraction efficiencies concluded that Lys and Met are the most limiting AA for lactating dairy cows (Nichols et al., 1998; Piepenbrink et al., 1999). The discrepancy between recommendations may be a function of methodological approach to establishing Lys and Met requirement and endpoint measure of sufficiency. Limiting determinations of Lys and Met sufficiency to milk protein yield does not fully depict the inherent post-absorptive AA utilization. Several biological models have produced their own analysis of Lys and Met requirements as shown in Table 2. Additions to biological models continue to re-define the recommendations for dietary Lys and Met content as well as trying to address synergistic effects of both AA and other dietary nutrients that may later post-ruminal AA supply. As inadequate post-ruminal AA supply arises from one or more AA being presented to tissues in insufficient quantities, protein synthesis is limited and on-farm profitability not fully maximized.

Table 2. Recommended dietary inclusion level of Lys and Met in ration evaluation programs that use differing biological platforms.

	Biological Platform		
	NRC (v2001)	CPM	CNCPS (v6.1)
Met, % of MP			
Optimal	2.4	2.6	2.4
Practical	2.2	2.4	2.2
Lys, % of MP			
Optimal	7.2	7.5	6.7
Practical	6.6	6.9	6.1
Lys:Met Ratio	3.0	2.9	2.8

Adapted from Whitehouse et al., 2010.

Limitations in Lys and Met supply for tissue and milk protein synthesis had driven research to determine AA sufficiency and associated efficiency of metabolism (Schwab and Ordway, 2004). Research establishing the profile of essential AA for milk protein synthesis has focused on Lys and Met, as His is thought to adequate in corn-based

rations commonly fed in the United States. To determine the optimal profile of limiting AA, AA extraction efficiencies, transfer efficiencies, and ratios of uptake to output of AA across tissues are often measured. Most determinations of Lys and Met sufficiency have been limited to milk protein yield and have not examined post-absorptive AA utilization in establishing AA requirements (Berthiaume et al., 2006) nor has there been attempts to link physiological and molecular indices of AA metabolism. Moreover, post-absorptive AA metabolism in tissues is sensitive to nutritional, physiological, and environmental factors (Lobley and Lapierre, 2003). Amino acids serve in a number of metabolic roles, including precursors for protein synthesis, and tissue specific AA utilization is a combination of AA oxidation and protein deposition (Lobley, 1992). Information on AA and their links between tissues and response to altered AA supply is critical to reduce AA catabolic losses and improve efficiency of N utilization especially when limiting AA are considered.

LYSINE

Data for rates and extent of Lys metabolism by liver and mammary have been identified as a prerequisite for increased precision in formulating diets for lactating dairy cows based on MP requirements (Hanigan et al., 2004b). There is also a need to determine physiological response to changes in Lys supply, focusing on the specific changes in Lys catabolism. Post-ruminal infusion of Lys has been used as an experimental method to provide additional absorbable lysine to the dairy cow but has yielded inconsistent effects for milk production and composition (Rulquin et al., 1993; Robinson, 2010). The lack of predictable response to Lys infusion suggests differences in the efficiency of post-ruminal Lys utilization. A portion of this inefficiency may be due to variations in hepatic Lys catabolism, mammary Lys extraction, conversion of Lys to milk protein, or a combination of these processes (Lapierre and Lobley, 2001; Lobley and Lapierre, 2003).

In a recent study (Tucker et al., 2012), post-ruminal Lys supply was increased through abomasal infusion of Lys and data on how mRNA transcripts for key genes in Lys and protein catabolism in liver and mammary tissue changed were measured. Milk protein percent increased by 7.5%, plasma Lys increased by 74%, and α-aminoadipic acid increased by 51% with post-ruminal infusion of 63 g/d Lys compared to 0 g/d (Table 3). Expression of aminoadipate semialdehyde synthase, ornithine transcarbamoylase, and argininosuccinate synthase mRNA did not differ with post-ruminal infusion of Lys. Mammary mRNA for major milk proteins and AASS were not affected by Lys infusion. Post-ruminal infusion of Lys resulted in an 86% greater increase in aminoadipate semialdehyde synthase mRNA in liver compared with mammary mRNA. These changes suggest hepatic Lys metabolism is not responsive to Lys supply and availability of Lys to extra-hepatic tissue may be determined by hepatic Lys metabolism.

Table 3. Least squares means for lactational performance and blood metabolites of early lactation dairy cows supplied increased lysine through abomasal infusion.

	Lys Infusion, g/d					
	0	9	27	63	SE	*P* - value
Milk Protein, g	773	805	829	805	28	0.14
Milk urea N, mg/dL	8.2	9.0	8.8	10.1	0.63	0.19
Plasma urea N, mg/dL	11.0	10.8	11.4	10.8	0.40	0.13
Plasma, μM						
Ala	224^{x}	181^{y}	219^{xy}	206^{xy}	13.2	0.06
Arg	47.1^{xy}	44.1^{x}	55.5^{y}	53.2^{xy}	3.7	0.08
His	24.4^{a}	16.5^{b}	18.4^{ab}	14.2^{b}	1.9	<0.05
Leu	177^{ab}	155^{a}	191^{b}	176^{ab}	7.9	<0.05
Lys	39.5^{a}	45.8^{ab}	60.7^{bc}	68.7^{c}	6.2	< 0.05
Met	44.7^{xy}	36.8^{x}	47.5^{y}	43.7^{xy}	2.9	0.09
α-aminoadipic acid	3.11^{a}	2.81^{a}	2.94^{a}	4.69^{b}	0.50	< 0.05

$^{a-c}$ Means within a row with different superscripts differ ($P \leq 0.05$). ^{x,y} Means within a row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

As technology for protecting Lys has proven difficult over the years, use of rumen protected Lys has been increasing over the last several years as the industry has realized that supplying adequate levels of lysine is critical to maintaining milk production. Research continues to investigate the fate of Lys in the dairy cow and how the dairy industry can best use this to support the health and well-being of the cow. Moreover, providing measures of Lys metabolism by liver may aid in targeting tissue specific use of AA-N and allowing for increased precision in diet formulation.

METHIONINE

Methionine supplementation has been previously reported to provide beneficial effects in lactational performance in dairy cows (Armentano et al., 1997; St-Pierre and Sylvester, 2005; Ordway et al., 2009). Moreover, decades of having a reliable rumen-protected source of Met has eased its inclusion into dairy rations. Methionine not only spares other essential AA but also serves as a methyl donor, a backbone for gluconeogenesis, stimulates triacylglycerol clearance from liver through the action of very low density lipoproteins (VLDL), and serves as a substrate for antioxidant reactions (Bauchart et al., 1998; Martinov et al., 2010). Providing empirical evidence on how Met benefits the dairy industry is needed in order to better predict whole animal responses and the benefits of Met supplementation.

In a cell culture study (Han et al., 2014), Met was supplied to cultured bovine mammary epithelial cells exposed to hyperthermia conditions. Supplying 60 mg/L Met increased viability of the bovine mammary epithelial cells while also hindering morphological damage to the cells induced by exposure to hyperthermia conditions. Moreover, Met reduced lactate dehydrogenase leakage from cells, a sign of reduced cytotoxicity induced by hyperthermia. Indicators of oxidative stress, malondialdehyde

and nitric oxide formation and nitric oxide synthase activity were reduced by Met supplementation suggesting that Met has greater modes of action, such as cytoprotective effects, than previously thought.

This is further supported by literature from monogastric research that supplemented Met to quails under acute heat stress (Del Vesco et al., 2014). Methionine supplementation to a Met deficient diet increased glutathione production. Greater glutathione production may be a direct response to increased presence of reactive oxygen species suggesting glutathione acts as an antioxidant. Moreover, Met plays a greater role in acting as an antioxidant through reacting with several reactive oxygen species to form methionine sulfoxide. Methionine sulfoxide can then be transformed back to Met in a pathway allowing cellular proteins to be protected from reactive oxygen species damage (Luo and Levine, 2009). Finally, concentrations of biological markers indicating extent of lipid peroxidation damage were similar among those quails under heat stress but supplemented Met and those quails kept in non-heat stress conditions. Though research specific to the protective action of Met under heat stress has not been shown in the whole cow model, data from cell culture and non-ruminant research suggests different modes of action for Met in addition to its ability to increase lactational performance.

Recent data (Osorio et al., 2013) suggests the Met should be supplemented during the transition period. Research has shown that Met supplementation during the transition period can improve milk and milk protein yield (St. Pierre and Sylvester, 2005). Increases in lactational performance in those cows supplemented Met are thought to be due in part to optimization of body lipid reserve that allow for positive energy balance to be achieved in less time than cows not provided Met. Moreover, those cows supplemented Met during the transition period had a lower potential to develop ketosis indicating that overall health of the cow was not impaired by Met supplementation. This potentially indicates altered hepatic lipid metabolism induced by Met (Osorio et al., 2013). Though the mechanism behind this alteration in hepatic metabolism is not clear due to failure to see sustained alterations in liver triacylglycerol, Osorio et al. (2013) suggests apolipoprotein B-100 synthesis may have a role due to altered concentrations with Met supplementation.

Together these data suggest that the role for Met in the dairy cow diets, lactating or not, extends beyond improving lactational performance. Research continues to investigate the fate of Met in the dairy cow and how the dairy industry can best use this to support the health and well-being of the cow. Improvements in our knowledge of how Met is utilized will serve to improve existing mathematical models and resulting predictions in regards to lactational performance.

FARM APPLICATIONS AND CONCLUSIONS

Supply of MP should be positive during lactation in order to optimize lactational performance. In addition, Lys supply should range from 6.1 to 6.7% of MP and Met supply should range between 2.2 and 2.4% of MP with particular focus on the early

lactation period. During the dry period, MP should be supplied at greater than 1200 g/d, while Lys should be supplied at 90 g/d and Met at 30 g/d (French, 2013). Supplying MP, Lys, and Met at these levels allows for optimal transition into lactation as well as maximum milk and milk protein yield.

Metabolizable protein and AA play a role in regulating lactational performance at all stages of lactation. New research suggests that Met has a function in hepatic lipid metabolism and protecting animals from heat stress while additional functions of Lys are still to be explored. As protein and AA metabolisms are an integral and dynamic portion of the physiology of dairy cows it is critical to ensure research progresses past gains in milk protein and toward identifying additional benefits to the cow that supports both overall well-being and health as well.

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