Advances in Animal Biosciences (2013), **4:s1**, pp 15–18 © The Animal Consortium 2013
doi:10.1017/S2040470013000265

advances in animal biosciences

Diet formulation as an effective tool for mitigating nitrogen excretion in dairy systems

A. N. Hristov†

Department of Animal Science, The Pennsylvania State University, University Park, PA 16802, USA

Nitrogen (N) losses and ammonia emissions from livestock operations represent a significant water and air pollutant. It has been repeatedly demonstrated that reduction in dietary protein input results in improved N utilization efficiency and reduced N losses with manure in dairy cows. Reducing dietary protein can also benefit the producer by reducing feed cost. These interventions, however, have to be balanced with the risk of decreased productivity. If animal requirements for metabolizable protein (MP) are not met, productivity cannot be sustained. Supplementation with rumen-protected (RP) amino acids (AA) limiting milk production and milk protein synthesis may compensate for the lack of MP in dairy cow diets. Long-term experiments at Penn State University demonstrated reduced urinary N losses, blood urea, milk urea N, and ammonia emissions from manure with MP-deficient v. MP-adequate diets. In some trials, however, milk production and milk protein yield were decreased with the MP-deficient diets. Supplementation of these diets with RPAA (Lys, Met and His) increased dry matter intake and milk yield of the cows to levels comparable with MP-adequate diets and can be a successful strategy for reducing N losses and maintaining productivity in lactating dairy cows fed low-protein diets.

Keywords: Nitrogen, Amino Acid, Dairy Cow

Introduction

Nitrogen (N) emissions from agriculture are a significant water and air pollutant and the role of livestock in N pollution has been known for decades (Viets, 1974). Nitrogen, along with phosphorus, is a major cause of water quality impairment (USEPA, 2009). In the USEPA report, 32% of US streams were rated poor in relation to N pollution and agriculture was the most important source of river and stream impairment. Crop production, animal feeding operations, grazing and manure runoff are the main sources of N pollution from agriculture (USEPA, 2009). Nitrogen excreted with feces or urine has a different fate and contributes differently to ground water nitrate and ammonia (NH_3) emitted from cattle manure. Lee *et al*. (2011a) demonstrated that more than 90% of NH_3-N emitted during the first 10 days of manure storage originates from urine (Figure 1).

Ammonia emitted from animal feeding operations is an environmental and human health hazard, contributing to eutrophication of surface waters and nitrate contamination of ground waters, soil acidity and fine particulate matter formation (USEPA, 2004a and 2004b). In addition, NH_3 emission is a net loss of manure fertilizer value to the producer. For example, daily NH_3-N losses of 25% to 50% of the N excreted in manure have been estimated using mass balance approaches for dairy cows and feedlot cattle (Hristov *et al*., 2011). Emitted NH_3 reacts quickly with atmospheric sulfuric and nitric acids to form fine particulate matter ($PM_{2.5}$), which according to the World Health Organization affects more people than any other air pollutant (WHO, 2005). Even low concentrations of air pollutants have been related to a range of adverse health effects (Oberdorster, 2000; Miller *et al*., 2007). It has been estimated that, across different regions of the United States and under different weather conditions, $PM_{2.5}$ formed from NH_3 emitted from livestock operations contributed on average from 5% to 11% of the total $PM_{2.5}$ concentrations, but could reach 20% in some regions and in cool weather (Hristov, 2011). Ammonia can have an impact on water quality at a considerable distance from the emission source. Models have predicted an average lifetime of atmospheric NH_3 aerosol of 3 to 4 days (Pye *et al*. 2009). Grimm and Lynch (2005), for example, estimated ammonium wet deposition in the Chesapeake Bay watershed at 2.6 kg/ha, or about 47% of the total inorganic N deposition.

The fact that urinary N is more labile than fecal N and is the primary contributor to NH_3 and total N losses from manure emphasizes the importance of reducing urinary N losses and/or shifting N excretion from urine to feces. The advantages of decreasing dietary crude protein (CP) for reducing manure N losses and gaseous N emissions from livestock operations have been demonstrated and

† E-mail: anh13@psu.edu

https://doi.org/10.1017/S2040470013000265 Published online by Cambridge University Press

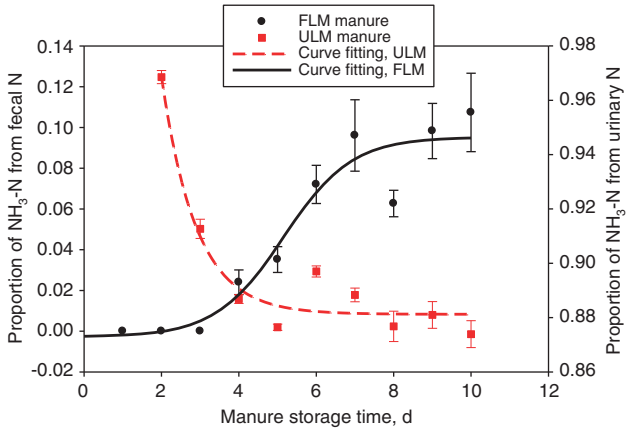


Figure 1 Proportion (means ± s.e.; $n = 8$) of NH_3-N emitted from manure originating from fecal- or urinary-N. Data derived from manure containing ^{15}N-labeled feces (FLM), or ^{15}N-labaled urine (ULM; Lee *et al.*, 2011a).

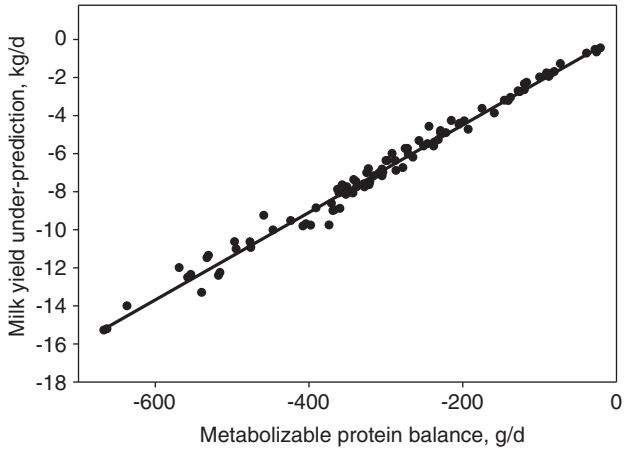


Figure 2 Relationship of metabolizable protein (MP) balance (NRC, 2001) and under-prediction of milk yield (NRC (2001) MP-allowable milk yield, kg/day – actual milk yield, kg/day) in dairy cows: milk yield under-prediction, kg/day = 0.0991 (±0.0905) + 0.0230 (±0.0003) × MP balance (g/day); $n = 97$ (MP balance, min = −20 g/day and max = −666 g/day), $R^2 = 0.99$, $P < 0.001$ (data from Lee *et al.*, 2012b).

discussed in various publications and reviewed by Hristov *et al.* (2011). It has been unequivocally shown that reduction in dietary N input can be a successful strategy for improving N utilization efficiency and reducing N losses with manure in dairy cows. Reducing dietary protein can also benefit the producer by reducing feed cost. There are numerous examples where decreasing protein concentration in diets for dairy or beef cattle dramatically decreased manure N losses without affecting animal production (see Hristov *et al.*, 2011). These interventions, however, have to be balanced with the risk of decreased productivity. If animal requirements for metabolizable protein (MP) are not met, production cannot be sustained. Milk production of high-producing dairy cows, for example, was reduced with diets containing around 14% CP (Lee *et al.*, 2011b; Lee *et al.*, 2012a). Production losses with low-protein diets can be caused by: (1) depressed dry matter intake (DMI) due to impaired rumen function or physiological regulation of intake, (2) deficiency of ruminally degradable protein (RDP) and/or (3) insufficient supply of key amino acids (AA) limiting milk protein synthesis. The effect of low-protein diets on feed intake is critical and must always be considered (Lee *et al.*, 2012a). In some cases, dietary CP as low as 12%, did not affect milk production of dairy cows, although nutrient digestibility and microbial protein synthesis in the rumen were depressed (Aschemann *et al.*, 2012). In the latter study, however, intake of the cows was restricted and the important effect of protein on DMI (Picard *et al.*, 1993; Lee *et al.*, 2012a) could not be demonstrated. Supplementation with rumen-protected AA (RPAA) limiting milk production and milk protein synthesis may compensate for the lack of MP in dairy cow diets. In some cases, this was a successful strategy (Leonardi *et al.*, 2003; Berthiaume *et al.*, 2006; Broderick *et al.*, 2008), but not in others (Socha *et al.*, 2005; Davidson *et al.*, 2008; Benefield *et al.*, 2009). This uncertainty of the impact of low-protein diets on milk production can jeopardize efforts for promoting environmentally sustainable practices to the dairy industry. Thus, it is critically important to elucidate the mechanisms by which dietary protein and post-ruminal AA supply regulate feed intake and milk production in dairy cows, so the effect of protein feeding on animal production can be successfully predicted.

Main findings

Inaccurate estimation of feed RDP, unaccounted physiological mechanisms, such as urea recycling (Huhtanen and Hristov, 2009), and improved efficiency of conversion of MP to milk protein (Doepel *et al.*, 2004) as diets become increasingly deficient in MP are likely responsible for the observed under-prediction of milk yield in cows fed MP-deficient diets in several experiments conducted at The Pennsylvania State University (Figure 2). In these experiments, urinary N losses, blood urea and milk urea N were consistently decreased compared with the control, MP-adequate diets (Lee *et al.*, 2011b, 2012a and 2012b). Manure from cows fed MP-deficient diets had up to 40% decreased NH_3-emitting potential (Lee *et al.*, 2012b). As indicated earlier, however, in some experiments, DMI, milk production and milk protein concentration were decreased with the MP-deficient diets. Supplementation with RPAA (Met and Lys) appeared to alleviate some of these negative effects, but the trends for depressed DMI and milk production still existed (Lee *et al.*, 2012b).

Analysis of the data from these long-term, continuous design studies showed a clear trend for decreased blood plasma His concentrations with the MP-deficient, compared with the control diets (Figure 3). Further, analysis of rumen bacterial samples from unrelated experiments conducted at Pennsylvania State University (with diets similar in feed ingredient composition to the diets fed in the low-protein experiments) indicated about 27% lower His than Met concentration in bacterial protein (Figure 4). Microbial protein is an increasingly important source of AA for the cow when MP-deficient diets are fed. Therefore, based on the plasma His and ruminal bacterial AA composition data, we hypothesized

https://doi.org/10.1017/S2040470013000265 Published online by Cambridge University Press

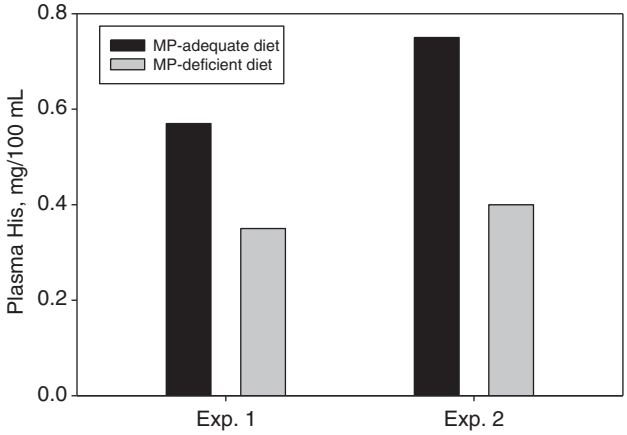


Figure 3 Effect of dietary metabolizable protein (MP) supply on blood plasma His concentration in lactating dairy cows (Exp. 1; s.e.m. = 0.053, $P = 0.001$; Exp. 2; s.e.m. = 0.042, $P < 0.001$). The MP-adequate diets met MP requirements of the cows and the MP-deficient diets were about 12% to 15% deficient in MP according to NRC (2001) (data from Lee *et al.*, 2012a and 2012b).

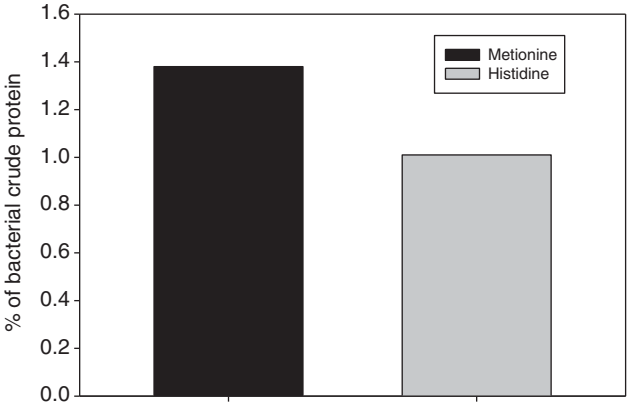


Figure 4 Methionine and histidine concentrations (% of CP) in fluid-associated ruminal bacteria isolated from lactating Holstein dairy cows ($n = 14$). Met, s.d. = 0.07; His, s.d. = 0.29; $P < 0.001$ (data from Lee *et al.*, 2012a).

that His may become a limiting AA in high-producing dairy cows fed corn silage- and alfalfa haylage-based diets deficient in MP. Indeed, His has been identified as the first limiting AA in lactating cows fed grass silage-based diets with low inclusion of plant protein supplements (Kim *et al.*, 1999; Vanhatalo *et al.*, 1999), but has not been implicated as a limiting AA in dairy cows fed typical North American, corn and alfalfa silage-based diets.

To test our hypothesis, we conducted a long-term (12 weeks), continuous design experiment with 48 lactating dairy cows (75 ± 5.6 days in milk). Following a 2-week covariate period, cows were blocked by stage of lactation and milk yield and randomly assigned to one of four diets, based on corn silage and alfalfa haylage: control, MP-adequate diet (AMP; MP balance: +9 g/day); MP-deficient diet (DMP; MP balance: −317 g/day); DMP supplemented with RPLys (AminoShure® -L; Balchem Corporation, New Hampton, NY, USA) and RPMet (Mepron®, Evonik Industries AG, Hanau, Germany; DMPLM); and DMPLM supplemented with an experimental RPHis preparation (DMPLMH; Balchem Corporation). CP content of the AMP and DMP diets was 15.7% and 13.5% to 13.6%, respectively. Apparent total tract digestibility of all measured nutrients, plasma urea-N and urinary-N excretion were decreased by the DMP diets compared with AMP. Milk N secretion as a proportion of N intake was greater for the DMP diets compared with AMP. Compared with AMP, DMI tended to be lower ($P = 0.06$) for DMP, but was similar for DMPLM and DMPLMH (Table 1). Milk yield was decreased by DMP, but was similar to AMP for DMPLM and DMPLMH, paralleling the trend in DMI. Milk fat and true protein content did not differ among treatments, but milk protein yield was increased by DMPLM and DMPLMH compared with DMP and was not different from AMP. Supplementation of the DMP diets with RPAA increased plasma Lys, Met and His concentrations. These data clearly identified His as a limiting AA in high-producing dairy cows fed corn silage and alfalfa haylage-based diets, deficient in MP. Milk N efficiency was increased and urinary N losses were dramatically decreased by the DMP diets. Based on our data and the similar concentration of Met and His in milk protein essential AA (5.5%, according to NRC, 2001), we concluded that MP should contain 2.2% His for lactating dairy cows fed corn silage and alfalfa haylage-based diets (similar to the recommended concentration of Met in MP; Schwab *et al.*, 2005).

Table 1 *Effect of metabolizable protein supply and rumen-protected amino acid supplementation on DMI and milk production and composition in dairy cows (data from Lee* et al.*, 2012a)*

	Diet					
Item	AMP	DMP	DMPLM	DMPLMH	s.e.m.	*P*-value
DMI (kg/day)	24.5	23.0	23.7	24.3	0.43	0.06
Milk yield (kg/day)	38.8[a]	35.2[b]	36.9[ab]	38.5[a]	0.74	<0.01
Milk fat (kg)	1.34	1.20	1.21	1.23	0.045	0.10
Milk true protein (kg/day)	1.13[a]	1.01[b]	1.10[a]	1.14[a]	0.025	<0.01

DMI = dry matter intake; AMP = MP balanced diet; DMP = MP-deficient diet; DMPLM = DMP supplemented with RPLys (AminoShure® -L) and RPMet (Mepron®); DMPLMH = DMPLM supplemented with an experimental RPHis product (Balchem Corp., NY).
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

Conclusions

Overfeeding of dietary protein results in increased urinary N losses and NH_3 emissions from manure and decreased milk N efficiency in dairy cows. Feeding MP below cow requirements, however, may result in decreased milk and milk protein yields, although milk production will be significantly greater than predicted by the NRC (2001) model. Based on experimental data, we hypothesized that His may be an AA limiting milk production in lactating dairy cows fed diets based on corn silage and alfalfa haylage. This hypothesis was tested in a long-term trial by supplementing an MP-deficient diet with RPAA, that is, Lys, Met and His.

https://doi.org/10.1017/S2040470013000265 Published online by Cambridge University Press

Data from this experiment showed increased DMI with RPAA supplementation, particularly when all three RPAA were supplemented. The increased DMI triggered milk and milk protein yield responses with the RPAA-supplemented diets. As total-tract fiber digestibility was suppressed with all MP-deficient diets, we propose that, similar to monogastric species, AA play a role in DMI regulation in dairy cows. Analysis of rumen bacterial samples indicated significantly lower His than Met concentration in bacterial protein. Microbial protein is an increasingly important source of AA for the cow when MP-deficient diets are fed and our data indicate that His may be a limiting AA in high-producing dairy cows fed corn silage- and alfalfa haylage-based diets deficient in MP. We propose that the proportion of His in MP should be similar to that of Met, which, for practical purposes, is suggested to be 2.2%. The MP-deficient diets in this experiment clearly increased milk N efficiency and decreased dramatically urinary N losses, which, based on previous observations, is expected to reduce manure NH_3 and potentially nitrous oxide emissions. Thus, supplementation of a MP-deficient diet with RPAA (Met, Lys and His) has the potential to reduce N losses and gaseous N emissions while maintaining milk production and milk protein yield in dairy cows.

Further information

The Dairy Solutions Symposium is a biennial event that covers a wide variety of themes and topics of relevance and importance to the dairy industry. The aim is to provide high level, up-to-date information and research to dairy professionals, technologists and scientists. In 2012, the theme addressed the biggest challenge facing all those involved in dairy production: optimizing production efficiency while lowering environmental impact. For more information, please visit www.dairycowsolutions.com or contact dairycowsolutions@alltech.com.

References

Aschemann M, Lebzien P, Hüther L, Döll S, Südekum KH and Dänicke S 2012. Effect of niacin supplementation on digestibility, nitrogen utilisation and milk and blood variables in lactating dairy cows fed a diet with a negative rumen nitrogen balance. Archives of Animal Nutrition 66, 200–214.

Benefield BC, Patton RA, Stevenson MJ and Overton TR 2009. Evaluation of rumen-protected methionine sources and period length on performance of lactating dairy cows within Latin squares. Journal of Dairy Science 92, 4448–4455.

Berthiaume R, Thivierge MC, Patton RA, Dubreuil P, Stevenson M, McBride BW and Lapierre H 2006. Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows. Journal of Dairy Science 89, 1621–1634.

Broderick GA, Stevenson MJ, Patton RA, Lobos NE and Olmos Colmenero JJ 2008. Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. Journal of Dairy Science 91, 1092–1102.

Davidson S, Hopkins BA, Odle J, Brownie C, Fellner V and Whitlow LW 2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. Journal of Dairy Science 91, 1552–1559.

Doepel L, Pacheco D, Kennelly JJ, Hanigan MD, López IF and Lapierre H 2004. Milk protein synthesis as a function of amino acid supply. Journal of Dairy Science 87, 1279–1297.

Grimm JW and Lynch JA 2005. Improved daily precipitation nitrate and ammonium concentration models for the Chesapeake Bay Watershed. Environmental Pollution 135, 445–455.

Hristov AN 2011. Contribution of ammonia emitted from livestock to atmospheric $PM_{2.5}$ in the United States. Journal of Dairy Science 94, 3130–3136.

Hristov AN, Hanigan M, Cole A, Todd R, McAllister TA, Ndegwa PM and Rotz A 2011. Ammonia emissions from dairy farms and beef feedlots: a review. Canadian Journal of Animal Science 91, 1–35.

Huhtanen P and Hristov AN 2009. A meta-analysis of the effects of protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92, 3222–3232.

Kim C-H, Choung J-J and Chamberlain DG 1999. Determination of the first-limiting amino acid for milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal. Journal of the Science of Food and Agriculture 79, 1703–1708.

Lee C, Hristov AN, Cassidy T and Heyler K 2011a. Nitrogen isotope fractionation and origin of ammonia nitrogen volatilized from cattle manure in simulated storage. Atmosphere 2, 256–270, doi:10.3390/atmos2030256.

Lee C, Hristov AN, Hyler KS, Cassidy TW, Long M, Corl BA and Karnati SKR 2011b. Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows. Journal of Dairy Science 94, 5544–5557.

Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, de Veth MJ, Patton RA and Parys C 2012a. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed metabolizable protein-deficient diet. Journal of Dairy Science 95, 6042–6060.

Lee C, Hristov AN, Heyler KS, Cassidy TW, Lapierre H, Varga GA and Parys C 2012b. Effects of metabolizable protein supply and amino acids supplementation on nitrogen utilization, production and ammonia emissions from manure in dairy cows. Journal of Dairy Science 95, 5253–5268.

Leonardi C, Stevenson M and Armentano LE 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. Journal of Dairy Science 86, 4033–4042.

Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL and Kaufman JD 2007. Long-term exposure to air pollution and incidence of cardiovascular events in women. The New England Journal of Medicine 356, 447–458.

National Research Council 2001. Nutrient requirements of dairy cattle, 7th revised edition. Natl Acad Sci, Washington, DC.

Oberdorster G 2000. Pulmonary effects of inhaled ultrafine particles. International Archives of Occupational and Environmental Health 74, 1–8.

Picard ML, Uzu G, Dunnington EA and Siegel PB 1993. Food intake adjustments of chicks: short term reactions to deficiencies in lysine, methionine and tryptophan. British Poultry Science 34, 737–746.

Pye HOT, Liao H, Wu S, Mickley LJ, Jacob DJ, Henze DK and Seinfeld JH 2009. Effect of changes in climate and emissions on future sulfate-nitrate-ammonium aerosol levels in the United States. Journal of Geophysical Research 114, D01205, doi:10.1029/2008JD010701.

Schwab CG, Huhtanen P, Hunt CW and Hvelplund T 2005. Nitrogen requirements of cattle. In Nitrogen and phosphorus nutrition of cattle and environment (ed. E. Pfeffer and A. N. Hristov), pp. 13–70. CAB International, Wallingford, UK.

Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab CG, Ducharme GA and Robert JC 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. Journal of Dairy Science 88, 1113–1126.

USEPA (U.S. Environmental Protection Agency) 2004a. National Emission Inventory – Ammonia Emissions from Animal Husbandry Operations. Washington, DC: USEPA.

USEPA 2004b. Air quality criteria for particulate matter. Volume I. Washington, DC: USEPA.

USEPA 2009. National Water Quality Inventory: 2004 Report to Congress. Washington, DC: USEPA.

Vanhatalo A, Huhtanen P, Toivonen V and Varvikko T 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. Journal of Dairy Science 82, 2674–2685.

Viets FG 1974. Fate of nitrogen under intensive animal feeding. Federation Proceedings 33, 1178–1182.

WHO (World Health Organization) 2005. Air quality and health. Retrieved September 19, 2012 http://www.who.int/mediacentre/factsheets/fs313/en/index.html.

https://doi.org/10.1017/S2040470013000265 Published online by Cambridge University Press