Nutritional and Genetical Factors Influencing Nitrogen Metabolism and Excretion in Dairy Cows: A Review

Daniel Cătălin Necula[1,2], Igori Balta[3,4], Nicolae Corcionivoschi[1,3], Lavinia Ştef[1*]

[1]Banat University of Animal Sciences and Veterinary Medicine "King Michael I of Romania", Faculty of Bioengineering of Animal Resources, Calea Aradului, 119, 300645-Timișoara, România
[2]Alltech Biotechnology Romania, 60102-București, România
[3]Agri-Food and Biosciences Institute, Bacteriology Branch, Veterinary Sciences Division, 18a Newforge Ln, BT9 5PX, Belfast, Northern Ireland, United Kingdom
[4]University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotechnologies, 400372-Cluj-Napoca, România

Abstract
This paper aimed to review the literature concerning nitrogen metabolism and excretion in dairy cows in the light of actual global climate change picture. Nutritional factors like dietary crude protein or dietary carbohydrate concentration have a significant effect on nitrogen balance, through the decreasing capacity of the total N excretion and improving the milk nitrogen efficiency. At the same time, the protein and carbohydrate degradability rates as well as dietary minerals will impact the excretion routes, urinary and faecal. Shifting between urinary to faecal pathway could benefit to the mitigation of air pollution as the faecal nitrogen content is less prone to volatilisation compared to the urine nitrogen. Feed additives such as direct feed microbial, plant secondary metabolites and rumen-protected amino acids can mitigate and shift the N excretion from the urinary to the faecal pathway. Finally, breeding animals for lower MUN traits could also be considered as an efficient approach for a longer-term strategy to reduce N emissions.

Keywords: dairy cow, metabolism, milk urea nitrogen, nitrogen efficiency, nitrogen emission, nitrogen excretion

1. Introduction

Nitrogen (N) emission from livestock production is a global concern and is accounted for one-third of human-induced nitrogen emissions globally [1]. The ruminants are playing an important role for the human food industry due to the ability to convert plant's low-quality nutrients that humans cannot use such as fibers/cellulose, into high-quality, dense nutrient food that humans can utilize.

Ruminants due to the complex fermenting processes of the stomach, the digestion of the feed differs from monogastric species. The difference is represented by the fermentation processes which occur in the non-glandular forestomach (rumen, reticulum and omasum) level providing a proportion of volatile fatty acids and microbial biomass.

However, the ruminants are not the most efficient in converting N into milk or meat. In dairy cows, the N efficiency (N milk/N intake) percentage varies from ≈ 15-35 [2, 3]. The rest is excreted into faeces and urine. The excretion of N in faeces is mainly in the form of organic compounds; meanwhile, the excretion in urine is in the form of urea. Under bacterial urease action, the urea is transformed in ammonia (NH_3) which volatilizes in the air, after that contributing to the air pollution [4, 5]. These losses contribute to environmental acidification, eutrophication which

* Corresponding author: Stef Lavinia
Tel. 0722351672, 0256277110
Email: lavi_stef@animalsci-tm.ro

negatively affects the biodiversity [5, 6]. From total livestock N emissions, the ruminants are accounted for ≈ 71 percent [1].

A better understanding is demanded, especially for the factors that influence N metabolism and excretion in ruminants to find appropriate solutions to increase N efficiency and attenuate faeces and urine excretions without affecting the animal performance.

Subsequently, a part of the carbohydrates and proteins from feed escapes the fermentative processes and are digested in the small intestine, similar to monogastric animals [7, 8]. Nitrogen balance or nitrogen efficiency parameters alongside the excretion of the N in urine (UN) and faeces (FN) are used to assess the N metabolism in dairy cows. Parameters like milk urea nitrogen (MUN), blood urea nitrogen (BUN) or plasma urea nitrogen (PUN) have been investigated since are more economically available markers for commercial dairy farms. Therefore, the scope of this paper is to review the factors that are influencing the N metabolism in dairy cows.

2. Materials and methods

Nutritional factors

Dietary protein concentration and rumen degradation rate

Dietary protein concentration has been shown to affect nitrogen metabolism in dairy cows. Olmos et al., found that as crude protein (CP) content of the diets increased over the 16.5%, BUN, MUN, and UN increased significantly. At the same time excretion of FN showed a linear and quadratic decline. Overall, there was a significant ($P<0.01$) decline in N efficiency (milk protein N/N intake), as protein concentration increased, from 36.5% for a diet with 13.5% CP to 25.4% for a diet with 19.4% CP. As the CP concentration of the ration increased, the percentage of N intake excreted in the urine increased significantly ($P<0.01$), going from 23.8% of N intake at 13.5% CP to 36.2% at 19.4% CP. The increase in UN excretion was mainly in form as urea N [9]. In a meta-analysis done by Huhtanen in 2009, N efficiency was greater for 16.5% CP than 17.8% CP diets by 12% [10]. Similar findings found Spek in his work, where N efficiency was greater for the diets with 16.1% CP compared with 17.1% CP by 5%, respectively [11]. More recently, Bahrami et al., decreased the CP concentrations through the reductions of the rumen degradable protein (RDP) fraction from the ration. Their study reduces the content from 10.9% up to 9.3% in dry matter (DM) base, meanwhile maintaining a constant level of rumen undegradable protein (RUP) of 5.5% DM across all treatments. Overall, the apparent N efficiency was greater ($P<0.01$) in favor of the ration with RDP=9.3% DM and the prediction of urinary excretion appeared significantly ($P<0.03$) lower [12].

It is noteworthy that the rate of protein degradation has also been found to influence N metabolism of dairy cows. Soluble intake protein (SIP) represents a fraction of rumen degradable protein, immediately available for rumen bacteria. Haig et al., tested the efficiency of three soluble crude protein concentrations on the total N balance. It was observed that as the dietary soluble crude protein increased the FN excretion declined in a significant manner ($P<0.05$), on the other hand, the parameter UN presented statistically significant increases ($P<0.05$). Interestingly that the N excreted in the milk was not affected ($P>0.05$). Overall, the manipulation of dietary SIP did not affected N efficiency but shifted to more N excretion via urine and lowered the FN output [13]. Broderick et al., achieved similar results in their experiment where animals received feed with similar levels of RDP, (10.5% DM), but with different degradation rates, respectively. These differences between degradation rate were achieved by replacing solvent treated soybean meal with urea and lignosulfonate treated soybean meal. As urea supplied a greater proportion of RDP, the MUN and BUN increased accompanied by higher excretion of urinary urea N (UUN) and a decreased excretion of FN. The N efficiency was not affected ($P>0.05$), except for a shift between urinary and faecal path was observed and was similar within the Haig et al., findings [14].

In conclusion, any increase on N intake in diets higher than 15-16% CP is going to decrease the N efficiency and it will increase the UN excretion, mainly as urinary urea. The reduction of protein degradability at rumen site is leading to a shift of N excretion from urinary to faecal path.

Dietary carbohydrates (CHO) and their degradation rate

During the fermentation process of the feed, the rumen bacteria utilize N and carbohydrates as well, resulting volatile fatty acids and microbial mass. The amount and their available speed rate have been shown to influence the ammonia uptake by ruminal bacterial species and which in turn could impact the N efficiency. The effect of dietary CHO on N metabolism in dairy cows was illustrated earlier by Morris et al. Their study investigated the effect of high starch (HS) against the high fat (HF) diet, formulated to be isoenergetic on N metabolism in Jersey cows. The UN excretion, as a proportion of N intake, tended to decrease (P<0.04) significantly in HS diet compared with HF (28.7 vs 33.3%). This improvement was accompanied by a significantly (P<0.01) greater N efficiency for HS diet (32.3 vs 28.8%), respectively [15].

Beside dietary amount, the degradation rate of CHO has been taken into consideration to affect N metabolism. According to Hristov et al., feeding with readily fermentable CHO decreased ammonia formation in the rumen section. Their study examined the effect of dextrose (GLU), starch (STA), fibre (NDF) and a mix of carbohydrates (MIX) on ruminal ammonia utilization in dairy cows. Ruminal ammonia concentration decreased by GLU and STA compared with NDF (P<0,001). The flow of microbial N formed was highest in the STA group compared with GLU and NDF (P=0.04 and 0.03) groups, correspondingly. Also, the administered GLU and STA lowered milk urea concentrations compared with NDF and MIX (P=0.002). Interestingly that urinary N loss was decreased (P=0.05) by GLU and STA, but the overall (faeces plus urine) N losses were not statistically affected (P=0.73) by treatments. That could happen because of higher FN excretion (P=0.002) in GLU and STA groups in comparison to NDF treatment. Overall N efficiency was not affected (P>0.05), respectively [16]. More recently, Sun et al., achieved a better N efficiency, but this was just due to similar milk true protein fraction combined with lower N intake in STA compared with GLU treatment [17]. Uddin et al., investigated the effects and interactions of diets formulated with two levels of forage NDF with either alfalfa silage (AS) or corn silage (CS) on N metabolism of Holstein and Jersey cows [18]. Cows fed with low forage NDF diet had lower UN excretion, but the milk N efficiency decreased, as a percentage from N intake, compared high forage NDF ration. In consequence the low forage NDF ration was less efficient regarding N metabolism resulting in a higher N balance, as a proportion from N intake. Comparing the NDF source, the CS-fed cow exhibited lower FN excretion but overall the N balance, as the proportion of N intake, was not significantly affected [18].

In conclusion the amount of dietary CHO in the ration is having positive effect on N efficiency and UN excretion. An increased in degradability rate of CHO could have an impact shifting the excretion from urinary to faecal path.

Water intake and dietary minerals

Water intake has been shown to influence N metabolism and excretion in dairy cows. There are not many investigations done on the water restriction effects in dairy cows. Although Burgos et al., measured, the N balance in milking cows challenged by 50% water restriction compared with *ad libitum* intake [19]. Expressed as a proportion from N intake, the excretion of milk and the UN was significantly (P<0.05) higher during water restriction than ad libitum period. Altogether their results showed that the nitrogen balance became negative during the water restriction period (P<0.05) [19].

Dietary minerals can influence water intake and urine output. Previously, Bannink et al., showed that minerals such as sodium (Na) and potassium (K) lead to an increase in water consumption and urine production in dairy cows [20]. Similar results were previously investigated by Spek when the dairy cows were fed with different levels of sodium chloride. The study found a significant linear effect (P<0.01) of Na intake and water consumption, including urine output [11]. The relationship between dietary minerals, water intake and N metabolism, have also been emphasized in that trial. PUN and MUN decreased significantly (P<0.01) as Na and water intake increased. Despite these changes, the N excreted in the milk was not affected. A significant shift from faeces to urinary N excretion has also been detected. Besides, the increase of N amount in urine was observed due to an increase of non-urinary urea nitrogen (NUUN) fraction of the urine. However, the UUN parameter has not been affected. That observation could be explained by the effect of increased water consumption in the rumen passage rate, and consequently, by an increased in microbial growth rate and outflow of microbial protein to the small intestine. This turned to an increase of derivates of absorbed microbial nucleic acids, resulting in an increased of NUUN. Overall, the N balance was similar across all of the treatments [11]. Similar to Na response, the K can affect the water intake and afterwards, the Nitrogen metabolism [20]. Cows fed with the rations based on grasses/legumes have a higher input of minerals, including K compared with the animals which diet was comprised of corn silage [21]. In the previous study Campeneere et al., found a significant (P<0.001) increase in urine production for the cows fed with 100% grass silage compared with the cows supplemented with the diet containing 100% corn silage. Interestingly to the fact that the urine volume was elevated (35 vs 14.4 L/day) and the UN excretion was not significantly altered. Moreover, despite the milk urea (MU) was significantly lower (P<0.001) in grass silage vs corn silage diet, the milk N excretion was not affected across treatments. Overall, this trial showed that there was no difference between the diets on the N balance[22].

In conclusion, water restriction may lead to a lower N balance and an improved N efficiency. The dietary minerals have a positive effect by lowering the MUN and PUN level. A shift between faecal to urinary paths of N has been spotted but, overall, N efficiency and N balance was not statistically affected as dietary minerals increase.

Additives

Many feed additives have been investigated aiming to improve N metabolism over the past decades, including direct feed microbials (DFM) or plant secondary metabolites (PSM). Mwenya et al., found that supplementation of DFM based on yeast culture (YC) combinations, significantly lowered the N excretion (P<0.05) in cows with the similar N intake [23]. Although the FN excretion was not statistically affected, the effect was potentiated by addition of galactooligosaccharides (GOS). The N excretion in urine decreased by 19% compared with YC and 22% compared with the control group. Interestingly that the cows which received supplementation with GOS only, did not respond in the same way [23]. Condensed tannins (CT) are PSM recognized to reduce the degradation of the protein at the ruminal level by increasing the undegradable fraction. These compounds are binding to the protein forming tannin-protein complexes which are stable at the pH of 3.5-8, respectively. At a lower pH, found in the abomasum; these complexes are dissociating releasing protein which is subjected to intestinal digestion and assimilation [24]. Recently, Zhang et al., investigated the response of tannins supplements on dairy cows [25]. The supplementation Bayberry tannins decreased the N excretion from urine as well as from faeces and significantly increased the nitrogen retention. In the same trial, the *Acacia mangium* and *Valonia tannins* treatments had a greater faecal excretion of N than in the control group, but with lower nitrogen retention [25]. More in-depth investigations are warranted as some examinations failed to demonstrate the efficacy of CT in this area [26, 27].

Rumen protected amino acids in conjunction with decreased protein concentrations have also been researched as a strategy to improve N metabolism. Lee et al., investigated the effect rumen-protected lysine, methionine and histidine on lactating dairy cows [28]. The experimental groups consist in four diets, one adequate (AMP) and three deficient (DMP) in metabolizable protein supply. From the DMP groups, one group was supplemented with rumen protected lysine and methionine (DMPLM) and one with rumen protected lysine, methionine and histidine (DMPLMH). The UN excretion was, on average, 36% lower (P<0.01) and was decreased (P<0.01) approximatively between 17 to 19% of N intake for the DMP diets compared with the AMP diet, which presented 23% of N intake. Simultaneously, the excretion of UUN was also reduced (P<0.01) by the DMP diets compared with AMP diet. Also, FN as the proportion of N intake was increased (P<0.01) by the DMP diets. Total N losses, including from faeces and urine were lower (P=0.01) for DMP and DMPLM compared with AMP, but demonstrated similar values between DMPLMH and AMP. Urinary allantoin and total purine derivates, and estimated microbial protein outflow from the rumen were not statistically affected by the treatments. The average of PUN was for 35% lower (P<0.01) for the DMP diets compared with the AMP diet supplementation [28]. More recently, another study investigated the effect of dietary protein level, and supplementation of rumen-protected lysine and methionine on lactating dairy cows. [29]. The experimental groups consist in four diets, one adequate (AMP) and three deficient (DMP) in metabolizable protein supply. From the

DMP groups, one group was supplemented with rumen protected lysine (DMPL) and one with rumen protected lysine and methionine (DMPLM). In contrast with the previous work, the total N excreted in faeces and secreted in milk, as a proportion from N intake, was not different among the experimental groups. Alternatively, the urinary N expressed as the proportion of N intake was lower for DMP diets compared with the AMP group, similar to the results from the previous findings. Urinary allantoin and total purine derivatives (allantoin+uric acid) excretion tended to decrease by 7 to 8% (P=0.06 to 0.08) for DMP and DMPLM compared with AMP and DMPL groups. PUN concentration was on average 23% lower (P=0.03) for DMP and DMPLM compared with AMP, respectively [29].

In conclusion feed additives may have the potential to mitigate and shift from the urinary to faecal path the N excretion.

3. Results and discussion

Genetic factors

Scientific achievements of recent decades in the fields of genetics concomitant with animal breeding led to the possibility to significantly increase the productivity of farm animals, efficiently improved the conversion ratio of feed and favoured rentable economic manufacture of livestock products. It is noteworthy to the fact that nitrogen concentrations from the urine samples involve difficult measurement operations, MUN being much easier to investigate comparably to the urinary or/and faecal N. According to up-to-date studies, which reported different genetic variables for the MUN heritabilities ranged between 0.14 to 0.59 alongside with increased coefficient variations offers a possible suggestion to induce changes or even manipulate with the MUN parameter via genetical selection [30-33]. Additionally, on a certain degree, the concentrations of MUN may also be dependent on the animals' individual genetic potential [32]. For example, the concentrations of MUN are lessened and are presumed to excrete reduced amounts of UN in the case of Jersey breeds in comparison to Holstein-Friesian cows. That is due to smaller anatomical body proportions and lower milking producing capacity attributed to Jersey cows [32].

The increased amounts of urea are usually interlinked with reduced fertility characteristics because of the toxic potential of urea and negative energetical balance. The obvious associations of MUN with the excretion of N in urine, faeces and milk set forward a possible and reasonable purpose that alleviation of the MUN concentrations would reduce environmental pollution with N which is possible with the precisely adjusted managemental practices implemented at the herd-level [30]. Notwithstanding, a plethora of circumstances may affect MUN parameter, and furtherly could lead to misinterpretations, therefore, essential investigation of possible factors in relationship with other non-genetic or genetic determinants, is required [34]. Previously studies reported that heritability status of the MUN is generally stable over different lactation periods [35]. However, that does not mean that the equivalent genes are acting throughout the entire lactation period, which indicates that the divergences may occur between the results in the studies. Previously, Buitenhuis et al., (2013) suggested that associations among single nucleotide polymorphisms (SNPs), heritabilities, correlations and variances could play as quintessential genetical key-indicators for the determination and consistent implementation of metabolic characteristics for cattle selection-breeding strategies. For example, an earlier study has reported the SNPs analysed in Italian Brown Swiss cows which were furtherly associated with MUN and where localised in the beta-2 adrenergic receptor (ADRB2), serpin peptidase inhibitor (PI) and SCD-1 [36]. The author suggested that these markers could improve genetically assisted selection programs by increasing their accuracy to achieve a better quality and health features of cattle.

Recent New Zealand studies have estimated that if the correlations between MUN and urinary nitrogen (UN) are keeping similar values after breeding programs, the excretion of UN for the offspring could be reduced by ≈ 6.6 kg/cow/annum only if the bulls with a decreased milk urinary nitrogen breeding values (MUNBV) are included in the program [32, 33]. Therefore, the application of such selection tools would generate future daughters with a reduced amount of MUN indices. At the same time, the study suggested that these models could potentially mitigate nitrogen pasture leaching by ≈20% for two decades alleviating the negative environmental impact. Moreover, a constant positive linear correlation amid MUNBV and urinary urea nitrogen (UUN) was recently described by Marshall et al., (2020), after the exploration of the two lactating stages and inclusion of two different diets. Of which the first dietary program was composed of *Lolium perenne* and *Trifolium pratense* and the second included *L. perenne* in combination with *Trifolium repens* and *Plantago lanceolata*, respectively [33]. Additionally, earlier review reported by Spek et al., (2013) indicated that MUNBV and UUN are not related to the efficiency of nitrogen utilisation, suggesting that more mechanistic approaches should be investigated for a more relevant correlation prediction among nitrogen related parameters [37].

Recent investigations from Northern Ireland reported the updated linear and multiple regression models for the prediction of the N excretion in the urine, faeces or complex manure using N intake as well as other related variables [38]. The study evaluated the datasets of Holstein-Friesian breed, including Holstein crossbreds from 1990 to 2019. The evaluation of the variance revealed that animals from the new set of data partitioned statistical significantly elevated fraction of consumed nitrogen in milk and appeared to excrete lesser proportions of N in urine and manure in comparison to cows from the old set of data. In the same vein, the analysis of linear regression of the new dataset displayed a lower positive slope in connection amid N intake and N excretion in total manure and urine as well as a higher positive slope was observed among N intake and milk N output relationship, when compared to the old dataset, respectively. Furthermore, the overall results of their study demonstrated that modern Holstein cows generations (high genetic merit) could utilize nitrogen from the feed in a more efficient manner than the cows from older populations (lower genetic merit) which were analyzed 15 years ago. Finally, modern dairy Holstein breeds managed with grassland-based dairy systems excreted decreased amounts of N in urine and faeces/kg of milk [38]. A similar genetical trend was observed in modern Brown Swiss sires which after 30 years of selection enhanced the qualitative and quantitative traits of milk as well as revealed to excrete lower MUN values and somatic cell score [39]. Moreover, Bobo et al., (2020) mentioned that heritability and the additive genetic parameters variations that co-exist in MUN and MUN-associated indices for nitrogen utilization efficacy could be modified via selection tools.

Nishimura specified that genetic factors are playing important roles concerning the genetical relationship analysis between fertility, MUN and digestive crude protein (DCP) fractions could probably enhance the accuracy for better fertilization, subsequently providing a suitable tool for the measurement of the metabolic problems [40]. According to the results effectuated on Polish Holstein-Friesian cattle, it was proposed that genetically yield traits, and somatic cell score correlations revealed a slightly increasing tendency towards milk urea (MU) after applying selection programs aiming to improve animals' udder health parameters [31]. To end up that section, it is noteworthy that farming indeed plays a significant role towards the control of phenotypic variances of MUN that are intimately dependent on herd-test-day (HTD) effects which could indicate that correctly adjusted dietary programs interconnected with proper managerial conditions are essentially necessary for the improvement of phenotypical datasets for genetic investigation of cattle breeds [41].

4. Conclusions

Reducing crude protein concentration is one of the most critical strategies that affect N metabolism and excretion. Increasing the high available CHO at the expense of high fat rations is another reliable approach. The synchrony between protein and carbohydrate fractions has impact of ruminal ammonia formation. Great care must also be taken in supplementing the ration with minerals, especially cations. This can lead to an increase in urine volume and UN excretion. Shifting between urinary to faecal excretion via dietary nutritional changes or supplementation with additives could benefit to the mitigation of air pollution as the faecal N content is not prone to volatilize compared to urine N. Breeding animals for lower MUN traits could be considered as an efficient approach for a longer-term strategy to reduce N emissions.

References

1. Uwizeye, A. et al., Nitrogen emissions along global livestock supply chains, Nature Food, 2020, 1(7), 437-446
2. Powell, J. M. et al., Validation of Feed and Manure Data Collected on Wisconsin Dairy Farms, Journal of Dairy Science, 2006, 89(6), 2268-2278
3. Gourley, C. J. P., Aarons, S. R. and Powell, J. M., Nitrogen use efficiency and manure management practices in contrasting dairy production systems, Agriculture, Ecosystems & Environment, 2012, 147, 73-81
4. Aguerre, M. J. et al., Effect of dietary crude protein on ammonia-N emission measured by herd nitrogen mass balance in a freestall dairy barn managed under farm-like conditions, Animal, 2010, 4(8), 1390-400
5. Erisman, J. W. et al., Consequences of human modification of the global nitrogen cycle. Philosophical Transactions of the Royal Society B: Biological Sciences, 2013, 368(1621), 20130116
6. Galloway, J. N. et al., Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions, Science, 2008, 320(5878), 889.
7. Krehbiel, C. R., INVITED REVIEW: Applied nutrition of ruminants: Fermentation and digestive physiology, The Professional Animal Scientist, 2014, 30(2), 129-139
8. Harmon, D. L., Nutritional Regulation of Postruminal Digestive Enzymes in Ruminants1, Journal of Dairy Science, 1993, 76(7), 2102-2111.
9. Colmenero, J. J. O. and Broderick G. A., Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows, Journal of Dairy Science, 2006, 89(5), 1704-1712
10. Huhtanen, P. and Hristov, A. N., A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows, Journal of Dairy Science, 2009, 92(7), 3222-3232
11. Spek, J. W., Variation of milk urea in dairy cattle: a study on factors that affect the relationship between urea concentration in milk and urea excretion in urine. 2013.
12. Bahrami-yekdangi, M. et al., Reducing crude protein and rumen degradable protein with a constant concentration of rumen undegradable protein in the diet of dairy cows: Production performance, nutrient digestibility, nitrogen efficiency, and blood metabolites, Journal of Animal Science, 2016, 94(2), 718-725
13. Haig, P. A. et al., Effects of Dietary Protein Solubility on Nitrogen Losses from Lactating Dairy Cows and Comparison with Predictions from the Cornell Net Carbohydrate and Protein System, Journal of Dairy Science, 2002, 85(5), 1208-1217
14. Broderick, G. A. and Reynal, S. M., Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows, Journal of Dairy Science, 2009, 92(6), 2822-2834
15. Morris, D. L. et al., Effects of high-starch or high-fat diets formulated to be isoenergetic on energy and nitrogen partitioning and utilization in lactating Jersey cows, Journal of Dairy Science, 2020, 103(5), 4378-4389
16. Hristov, A. N. et al., Effect of carbohydrate source on ammonia utilization in lactating dairy cows, Journal of Animal Science, 2005, 83(2), 408-421
17. Sun, F., Aguerre, M. J. and Wattiaux, M. A., Starch and dextrose at 2 levels of rumen-degradable protein in iso-nitrogenous diets: Effects on lactation performance, ruminal measurements, methane emission, digestibility, and nitrogen balance of dairy cows, Journal of Dairy Science, 2019, 102(2), 1281-1293
18. Uddin, M. E. et al., Enteric methane, lactation performances, digestibility, and metabolism of nitrogen and energy of Holsteins and Jerseys fed 2 levels of forage fiber from alfalfa silage or corn silage, Journal of Dairy Science, 2020, 103(7), 6087-6099
19. Burgos, M. S., et al., Effect of water restriction on feeding and metabolism in dairy cows, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2001, 280(2), R418-R427.
20. Bannink, A., Valk, H., and Van Vuuren, A. M., Intake and Excretion of Sodium, Potassium, and Nitrogen and the Effects on Urine Production by Lactating Dairy Cows, Journal of Dairy Science, 1999, 82(5), 1008-1018
21. Kappel, L. C. et al., Seasonal Changes of Mineral Content of Southern Forages, Journal of Dairy Science, 1985, 68(7), 1822-1827
22. Campeneere, S., Brabander, D. and Vanacker, J., Milk urea concentration as affected by the roughage type offered to dairy cattle, Livestock Science - LIVEST SCI., 2006, 103, 30-39
23. Mwenya, B. et al., Effects of Yeast Culture and Galacto-Oligosaccharides on Ruminal Fermentation in Holstein Cows, Journal of Dairy Science, 2005, 88(4), 1404-1412
24. Frutos, P. et al., Review. Tannins and ruminant nutrition, Spanish Journal of Agricultural Research, 2004, 2
25. Zhang, J. et al., Effect of Different Tannin Sources on Nutrient Intake, Digestibility, Performance, Nitrogen Utilization, and Blood Parameters in Dairy Cows, Animals, 2019, 9(8), 507.
26. Aguerre, M. J. et al., Effect of quebracho-chestnut tannin extracts at 2 dietary crude protein levels on performance, rumen fermentation, and nitrogen partitioning in dairy cows, J Dairy Sci., 2016, 99(6), 4476-4486
27. Benchaar, C., McAllister, T. A. and Chouinard, P. Y., Digestion, ruminal fermentation, ciliate protozoal

populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extracts, J Dairy Sci, 2008, 91(12), 4765-77
28. Lee, C. et al., Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet, Journal of Dairy Science, 2012, 95(10), 6042-6056
29. Lee, C. et al., Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows, Journal of Dairy Science, 2015, 98(3), 1885-1902
30. Stoop, W. M., Bovenhuis, H. and van Arendonk, J. A. M., Genetic Parameters for Milk Urea Nitrogen in Relation to Milk Production Traits, Journal of Dairy Science, 2007, 90(4), 1981-1986
31. Rzewuska, K. and Strabel, T., Genetic parameters for milk urea concentration and milk traits in Polish Holstein-Friesian cows, Journal of applied genetics, 2013, 54(4), 473-482
32. Beatson, P. R. et al., Genetic variation in milk urea nitrogen concentration of dairy cattle and its implications for reducing urinary nitrogen excretion, Animal, 2019, 13(10), 2164-2171
33. Marshall, C. J. et al., Grazing dairy cows with low milk urea nitrogen breeding values excrete less urinary urea nitrogen, Science of The Total Environment, 2020, 739, 139994
34. Rzewuska, K. and Strabel, T., Effects of some non-genetic factors on concentration of urea in milk in Polish Holstein-Fresian cows, Journal of Animal and Feed Sciences, 2013, 22 (3), 197-203
35. Buitenhuis, A. J. et al., Estimation of genetic parameters and detection of quantitative trait loci for metabolites in Danish Holstein mil, Journal of Dairy Science, 2013, 96(5), 3285-3295
36. Cecchinato, A. et al., Candidate gene association analysis for milk yield, composition, urea nitrogen and somatic cell scores in Brown Swiss cows, Animal, 2014, 8(7), 1062-1070
37. Spek, J. W. et al., A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle, The Journal of Agricultural Science, 2013, 151(3), 407-423
38. Chen, X. et al., Modern Holstein-origin dairy cows within grassland-based systems partition more feed nitrogen into milk and excrete less in manure, Science of The Total Environment, 2020, 727, 138561
39. Bobbo, T. et al., Short communication: Genetic aspects of milk urea nitrogen and new indicators of nitrogen efficiency in dairy cows, Journal of Dairy Science, 2020, 103(10), 9207-9212
40. Nishimura, K., Miura, S. and Suzuki, M., Genetic Relationships between MUN, and Predicted DCPun in Hokkaido Holstein Cows, Asian-Australas J Anim Sci., 2005, 18(9), 1209-1216
41. Roveglia, C. et al., Phenotypic analysis of milk composition, milk urea nitrogen and somatic cell score of Italian Jersey cattle breed, Italian Journal of Animal Science, 2019, 18(1), 405-409