Milk Urea Nitrogen as an Indicator of Nitrogen Metabolism Efficiency in Dairy Cows: A Review

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ABSTRACT

The milk urea nitrogen concentration can be used as a tool management of the nutritional strategies in dairy farm and of improving proteins utilization efficiency by dairy cows. The level of dietary crude protein is the most nutritional factor that influence the milk urea nitrogen concentrations in lactating cows. Recent researches suggest that the milk urea nitrogen content depends mainly on the nitrogen/energy ratio in the diet, but other many factors may affect ureogenesis. Level and quality of protein contents, milk yield, season effects or lactation stage, parity and lactation number, weight and breed, feeding frequency and water intake are factors associated with the variation of milk urea concentration. Several studies have suggested that measuring milk urea nitrogen may serve as indicator to monitor nitrogen efficiency in dairy cows and to improve milk nitrogen production. However, the targeted milk urea nitrogen values for optimizing the nitrogen utilization efficiency are different from those required for milk protein utilization efficiency is below this level (<11 mg/dL). Normal MUN values range from 10 to 14 mg/dL, but for many countries, the recommended milk urea nitrogen values for cow's milk are ideally ranged from 10 to 16 mg/dL of milk. Finally, to ensure a balance between milk protein production and reducing urea nitrogen excretion in urine and milk, recent studies suggest to include 16.5% of crude protein supply in dairy cows diets.

Keywords: Milk urea nitrogen, crude protein, dairy cow, nitrogen efficiency, urea nitrogen excretion

Urea is a main product of nitrogen metabolism synthesized in the liver from excess ammonia product derived from rumen-degraded proteins, digestible proteins in the small intestine and amino acids catabolized in different parts of the body and/or during glycogenesis processes in the liver (Schepers and Meijer, 1998). The urea secreted by the liver diffuses into blood, gains blood circulation and then excreted mainly asurine. The urea concentration tends to balance with blood and others body fluids such as saliva, genital secretions and milk (Butler, 1998). This balance reveals a positive correlation between the urea concentration in blood and urea in milk (Broderick and Clayton 1997, Kauffman and St-Pierre, 2001).

In recent years, milk urea nitrogen (MUN) concentration arouse an interest in evaluating dietary strategies, dairy cow's fertility (Butler *et al.*, 1996) and provides an opportunity to monitor the nitrogen used for milk protein production or rejected in the environment (Nousiainen *et al.*, 2004). Moreover, several authors reported relationships between dietary crude protein level, milk production and the

efficiency of the reproductive potential. Thus, high dietary protein intake stimulates milk production but may affect cow's fertility in some situations (Jordan and Swanson, 1979; Kaim *et al.*, 1983; Canfield *et al.*, 1990).

On the environmental side, Kohn *et al.* (2002) have reported a close relationship between the level of crude protein in the ration and the amount of nitrogen released into the environment. In this way, 75-85% of the excess proteins consumed by a dairy cow are excreted in urine, feces and milk (Kohn *et al.*, 2002; Spek, 2013). Up to 50% excess of urea synthesized in the liver is excreted from body cow via urine (Tamminga, 1992) and accounts for almost 70-80% of the total volume urinary nitrogen (Spek, 2013). Thus, the MUN concentration gives more information to alert on nutritional drifts in dairy farms.

In particular, high or low levels of MUN concentrations indicate an inefficiency in the using of dietary proteins due to an imbalance between proteins and energy (Royet al., 2003). The variation in the MUN content is correlated with several nutritional factors which the most important are the protein-energy balance in the diet of lactating cows (Oltner and Wiktorsson, 1983), the proteins degradability in the rumen and the availability of ammonia in the rumen in relation of rumen microbes capacity to capture it (Roseler et al., 1993). The highest MUN levels indicate excess degradable proteins in the rumen or an imbalance between the degradable and non-degradable protein associated at an energy deficiency in the diet (Geerts et al., 2004).

In contrast, lowest MUN concentrations are revealing deficiency of the protein supply, low protein degradation or a diet containing high proportion of ferment as cibles carbohydrates compared to degradable proteins available (Bruning – Fann, 1993).

In particular, monitoring milk urea is easily achievable compared to blood urea (Butler *et al.*, 1996) and is very important for formulation of the optimal dietary that can lead to an improvement of milk production, reproduction, reducing costs feeds and potential nitrogen losses in the environment (Dufrasne *et al.*, 2010). Measurements of MUN variation should be considered as a management parameter to improve the dietary strategies of a dairy herd.

PRACTICAL IMPLICATIONS OF MUN

Definition

Milk proteins are composed of two major fractions of the total nitrogen: true proteins and non-protein nitrogen (NPN). The true protein represents 95% of the total nitrogen in milk and consists of 80% casein and 20% of whey (Roy *et al.*, 2003). The NPN percentage in the total nitrogen of milk is usually ranging between 5 and 6% (Nickerson, 1960; Burton, 1967). In the NPN fraction itself, urea contributes between a third and half of the nitrogen (Shahani and Sommer, 1951). Milk urea accounts for 2.5 to 3.0% of total protein in cow's milk (Spek, 2013).

Physiological basis of the MUN

Urea is a nitrogenous compound synthesized in the liver from the excess ammonia, produced by breakdown of protein and non-protein nitrogen (NPN) in the rumen and from the excess amino acids catabolized in the liver. The overproduction of ammonia in the rumen is the main cause of urea transfer in the blood (Roy et al., 2011). In additional, the catabolism of the amino acids and excess peptides in different parts of the body contribute to increase the urea flow into the portal blood (Royet al., 2011; Huntington et al., 2000). Thus, ammonia in the portal blood can be derived from the deamination of amino acids absorbed in the gut varying between 27% and 51% (Parker et al., 1995). The excess of ammonia not used by the microbes for the synthesis of microbial proteins in rumen, diffuses passively into the blood via the rumen's wall to be converted into urea by the liver. The synthesis of urea by the liver is a means of detoxifying circulating blood ammonia (Spek *et al.*, 2012).

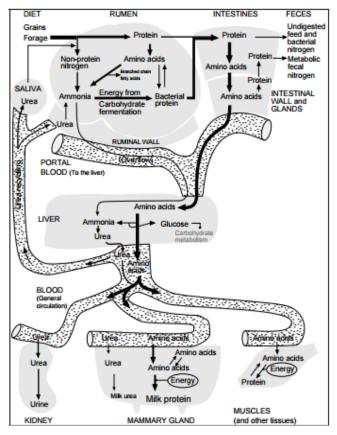


Fig. 1: Overview of nitrogen metabolism in dairy cows (Wattiaux, 2014).

The synthesized urea passes into the hepatic sinuses to join the circulatory system and is filtered from the blood by the kidney before being excreted from the body in urine (Swenson and Reece, 1993). By simple diffusion, urea drain into the mammary gland where it is an integral part of the NPN components of milk. Due to the physiological process of the urea cycle in mammals, the MUN concentration equilibrates with body fluids and is proportional to the concentration of blood urea (Roseler *et al.*, 1993).

This allows MUN to be considered as effective tool for managing nitrogen nutrition in lactating cows (Roy *et al.*, 2011), for reducing the amount of nitrogen lost in the urine and for optimizing

the efficiency of the use of nitrogen (Jonker et al., 1998)

Emergence circumstances of urea in milk

The excess degradable protein in the rumen or energy deficiency in the diet increase uremia and thus the level of milk urea (Arunvipas et al., 2003). On the other hand, a low level of urea in milk results from high proportion of fermentable carbohydrates compared to degradable proteins content in diet, which decrease ammonia production, reflecting the inadequate synthesis of microbial proteins in the rumen (Bruning - Fannand Kaneene, 1993). In milk, urea is a major component of NPN milk which can range from 5 to 30 mg per 100 g of cow's milk (Shahani and Sommer 1951). It should be noted that variations in the NPN content of milk are mainly related to the changes in the concentration of urea in milk (Journet et al., 1975, Roseler et al., 1993).

Relationship between milk and blood urea nitrogen

Several authors have showed that there is a positive correlation between urea blood and MUN concentrations (Doska et al., 2012). Urea is water-soluble molecule and when is present in circulatory system, diffuses into the body's aqueous organs such as udder and other genital glands (Butler et al., 1996). In addition, the epithelial cells of the mammary gland are very permeable to urea, so that a very close connection is observed between the urea content of the blood and that of the milk (Journet et al., 1975). Thus, measurement of MUN concentrations from normal milking is an easy assessment ofurea blood concentration (Lefebvre et al., 1995). According to various authors the urea blood concentration is reflected in milk from 83 to 98% as showed in the following table.

During lactation, the dietary demand for nitrogen compounds is critical because the secretion of milk proteins is higher, thus increasing blood urea concentrations and consequently milk urea concentrations (Doska *et al.*, 2012).

Authors	Correlation degree
Erbersdobler et al. (1980)	0,97
Oltner et al. (1985)	0,91
DePeters and Ferguson (1992)	0,88
Harris (1995)	0,83 - 0,98
Hutjens and Barmore (1995)	0,83-0,98
Lefebvre (1996)	0,83-0,98
Broderick and Clayton (1997)	0,95

The synthesis of milk proteins as well as the catabolism of excess amino acids are accompanied by high consumption of energy and take account of the link between blood urea and milk (Roseler *et al.*, 1993). The urea concentration in milk could be used as an indicator of the protein/energy balance of lactating cows (Jonker *et al.*, 1998; Godden *et al.*, 2001).

Advantages of milk urea estimation over blood urea

In comparison with urine or blood, milk is a stable fluid in which the urea dosage is more reliable (Kauffman and St-Pierre, 2001; Kohn *et al.*, 2002). The fact that urea equilibrates with all body fluids, make milk urea concentration to be similar to urea in blood and then be understood as a marker of urea nitrogen status in dairy farm (Roy *et al.*, 2003). As result, the dosage of urea in milk sample is easy to estimate than urea in the blood.

Milk urea as an indicator of nitrogen efficiency

One of the major challenges in nitrogen nutrition of dairy cows is to ensure the conversion of nitrogen into milk proteins. For dairy cows, the efficiency of conversion of nitrogen to milk proteins is low because more than 70% of ingested nitrogen is excreted as fecal or urinary nitrogen (Castillo *et al.*, 2001). According to Wattiaux and Crump (2014), one of methods to check nitrogen efficiency utilization is to

determine the ratio of total nitrogen excreted in the milk and nitrogen ingested by cows. The more efficient is obtained when the ratio is high. A study showed that 20-35% of ingested nitrogen is excreted as protein in milk and that up to 50% of consumed nitrogen is found in the urine (Tamminga, 1992). In principle, excess nitrogen in the dietary implies economic losses for the producer and increases the environmental cost and, in addition, can impair the performance of animals by reducing fertility and milk production of cows (NRC, 2001). The main factor affecting nitrogen utilization efficiency is the level of crude protein in the ration and reflect quantity and quality of proteins offered to dairy cows (Broderick, 2003). The importance of the crude protein levels in the ration on the nitrogen excretion in milk, feces and urine is crucial.

According to Colmenero and Broderick (2006), a ration containing 16.5% of crude protein appears to be ideal for maintaining milk production and minimizing nitrogen excretion in urine. The same authors reported that an increase of crude protein levelin the ration from 16.5 to 19.4% resulted in decreased nitrogen efficiency utilization of 5.4%. Under the conditions of their study, yield of milk and protein were not increased by feeding more than 16.5% crude protein. There were no significant difference between crude protein levels of 16.5% and 18% in terms of milk protein production, but the linear increase in nitrogen excreted in urine were very high for the 18% CP level and resulted from a sharp decline in nitrogen efficiency as dietary crude protein content increased (Colmenero and Broderick, 2006). Practically, monitoring the changes of MUN concentration is a good parameter to assess the nitrogen efficiency of dairy cows (Nousiainen et al., 2004). Nevertheless, the values of MUN concentrations to be targeted to optimize the nitrogen efficiency does not coincide with those sought for the production of milk proteins.

So, an increase in milk protein production can be achieved beyond of 11 mg/dl MUN values, while nitrogen efficiency is achieved at much lower values of these MUN levels (Nousiainen *et al.*, 2004).

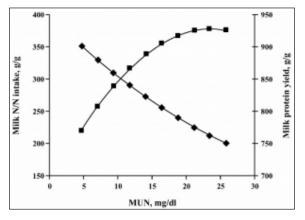


Fig. 2: Association between the efficiency of dietary N for milk protein production (\blacklozenge) and milk protein yield (**I**) with MUN (Nousiainen *et al.*, 2004).

Typical concentrations of urea in milk

Milk urea concentrations are usually expressed either as percentage or as mg/dL or as mmol/ litre of milk. The recommendations regarding the optimum milk urea concentrations vary according to the region or socio-economic considerations of each country (Arunvipas et al., 2004). During the 1960 years, the optimum of Milk Urea Nitrogen (MUN)was initially fixed at 10 mg/dL (Ide et al., 1966). From 1990, due to the intensification of nitrogen feeding systems to increase dairy production, the US set a margin of 10 to 16 mg/dL (Jonker et al., 1998). Sweden and others Nordic countries authorized a margin of 10 to 15 mg/dL (Carlsson and Pehrson, 1993). For Canada, MUN levels of 10 to 14 mg/dL represented an ideal range of nitrogen efficiency utilization by the dairy cow (Arunvipas et al., 2004). According to the current literature, normal MUN values ranged from 10 to 14 mg/dL (Jonker et al., 1998; Johnson and Young, 2003; Rajala-Schultz and Saville, 2003).

Concretely, MUN level values above or below the extremes are related to the inefficiency of nitrogen use by cows (Roseler *et al.*, 1993), which is mostly associated with a decline of their productive and reproductive performance (Bishonga *et al.*, 1994). For example, the MUN concentrations above 19 mg/dL were usually associated with decrease (P < 0.02) in conception rates (Butler *et al.*, 1996).

NUTRITIONAL FACTORS INFLUENCING THE MUN

Supply of nitrogen compound

The level of crude protein in the diet is positively correlated with the MUN concentration (Broderick and Clayton, 1997). One of the reasons for this correlation is the difficulty in meeting the energy requirement of high yielding cows and the farmer's habit to increase the protein intake for this category of cows (Rzewuska and Strabel, 2013). The efficacy of use of dietary proteins is function of the quality of protein ingested and depends in part on the amount of ammonia produced in the rumen (Roy *et al.*, 2011). Rook (1961) demonstrated that excess protein intake does not affect composition and milk production, but increases significantly the NPN content in milk.

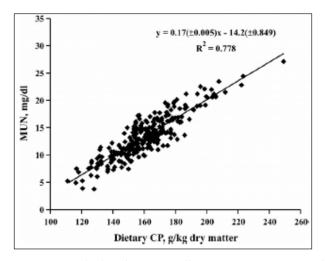


Fig. 3: Association between dietary CP content and MUN (Nousiainen *et al.*, 2004)

Thus, increasing the crude protein in the diet stimulates the milk proteins secretion

Online ISSN: 2277-3371

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without affecting significantly the true protein milk content. The diet rich in degradable proteins or soluble nitrogen in rumen and not contain sufficient amounts of non-structural carbohydrates result in increased urea milk concentration (Dufrasne *et al.*, 2010). According to the authors, Jonker *et al.* (1998), Kauffman and St-Pierre (2001) and Nousiainen *et al.* (2004), an increase of dietary crude protein is be accompanied by an increase in MUN and alsoresults in an increase of the nitrogen released meanly in urine.

In some cases, a lower level of crude protein $(\leq 12\%)$ in the diet can not affect milk production, although digestibility and microbial protein synthesis in the rumen are depressed (Aschemann *et al.*, 2012).

Energy level of the ration

The ruminal bacteria require energy to capture ammonia and use it as a source of nitrogen in order to grow (Nocek and Russell, 1988). The extent to which ammonia is used to synthesize microbial protein is largely dependent upon the availability of energy generated by the fermentation of carbohydrates (Wattiaux, 2014). Thus, the carbohydrates intake from the ration is an important factor in the synthesis of microbial proteins that determines the profile of amino acids produced during the proteolytic phase in the rumen (Hristov et al., 2004). The source of energy substrate influences the efficiency of microbial growth. Kyoung et al. (1999) found an increase in microbial synthesis of up to 29 g/kg of carbohydrate supplements when dairy cows were fed grass silage sprayed with sucrose solution.

On the average, 20 grams of bacterial protein is synthesized per 100 grams of organic matter fermented in the rumen. Bacterial and the percentage of protein in bacteria varies from 38 to 55% (Wattiaux, 2014). Sairanen *et al.* (2005) observed a linear increase in absorbable microbial proteins in the intestine when the cows on pasture have received a cereal-based energy supplement. The same authors showed that an increase in the protein content of milk is accompanied by a decrease in the level of milk urea.

However, Kebreab et al. (2002) reported that an increase in energy intake of 7 to 11 Mega joules/ kg DM of the diet leads to an increase in fecal nitrogen excretion and a reduction in excretion of urea in milk. This could be explained by the fact that an additional supply of energy causes a significant reduction in microbial growth probably due to the decrease of the ruminal pH (Broderick, 2003; Sairanen et al., 2005). Conversely, an inadequate intake of fermentable carbohydrates in the ration rich in degradable proteins leads to an accumulation of ammonia in the rumen that exceeds the bacterial capacity to capture it and thereby increases the flow of urea in the blood (Geerts et al., 2004; Arunvipas et al., 2004).

Non-protein nitrogen supply: Urea

Several sources of Non-Protein Nitrogen (NPN) are used in feeding of ruminants as protein correctors in nitrogen-deficient of foodstuffs. For several years, urea has been widely used as a substitute for preformed ruminant feed proteins (Wanapat et al., 2011). Thus, the addition of urea in a diet aims to produce ammonia necessary for the biosynthesis of microbial proteins. Nevertheless, its use must require certain precautions because its hydrolysis to NH₃ by the microbial enzymes of the rumen (Van Der Hoek, 1998), leads to an overproduction of ammonia which exceeds its absorption and subsequently the synthesis of the bacterial proteins gives way to excretion of urea in the urine (Highstreet et al., 2010). Some studies have shown that the use of NPN as a protein source can only be necessary if a considerable source of rapidly fermentable carbohydrates is available to optimize the growth of microbes in the rumen (Burroughs et al., 1975). Particularly, the growing microbial population uses ammonia to synthesize their proteins, decreases ammonia in the rumen and therefore decreases the flow of ammonia to the bloodstream (Bartley et al., 1976). In practice, it is recommended to incorporate in the ration an amount of urea providing less than 35% of the total nitrogen and this must represent less than 1% in terms of dry matter in the total ration (Poncelet, 1983). Bach et al. (2005) suggested not to exceed 0.5% urea in all diets considered low in protein but rich in starch. Consequently, Meggison et al. (1979) observed improved synthesis of microbial proteins by continuously and periodically distributing energy-rich rations containing urea or by using other NPN sources with low urea solubility. For, Salter et al. (1983), there was no interest to add the urea to nonmixed ration when an energy supply of 40% was distributed in two meals.

Synchronism between protein intakes and energy in the rumen

The synchronization between protein intake and energy is an important factor in improving the use of degradable proteins in the rumen (Huntington *et al.*, 2000). Bacterial growth requires a large amount of energy in synchrony with the rate of protein degradation in the rumen. High levels of rapidly degradable proteins lead to the overproduction of ammonia in the rumen, which requires sufficient energy to be captured by microbes (Tamminga, 1992).

Moreover, some authors reveal that the lack of synchronization in protein degradation and fermentation of carbohydrates, leads to the accumulation of ammonia in the rumen and storage of urea in the aqueous spaces of the body (Manson And Leaver, 1988), and may lead to potential deterioration in the reproductive efficiency, health and welfare of the animal. Enjalbert (2006) and Kolver *et al.* (1998) found that supplementation of non-structural carbohydrates synchronized with pasture have reduced ammonia in rumen from 22 to 43% in 3 to 5 hours after ingestion of food at grazing.

Compared to these results, (Taweel *et al.*, 2006) hypothesized that rumen microorganisms may have benefited a sufficient amount of energies in a contiguous and simultaneous manner to produce ammonia, so as to reduce the gap in energy supply following fermentation of grass fibers ingested during grazing time. If energy is not sufficient in the rumen or if the rates of fermentation of carbohydrates and protein degradation are not synchronized, the excess ammonia will pass through the rumen wall and be transported to the liver via the portal vein where ammonia is converted to urea. This process is the most important source of nitrogen loss for the animal and environmental pollution, because 40-60% of the urea produced in the liver is excreted in the urine (Huntington, 1989).

NON-NUTRITIONAL FACTORS INFLUENCING THE RATE OF MILK UREA

Several non-nutritional factors may modify the concentrations of urea in milk (Carlsson *et al.*, 1995). The most important of these factors are live weight and breed, frequency of milking and feeding, the lactation stage, month and season, age and parity, yield milk and milk protein production (Godden *et al.*, 2001).

Live weight and breed of animal

Kauffman and St-Pierre (2001) have shown that there is a positive correlation between live weight, animal breed and MUN concentration. According to (Kohn *et al.*, 2002), body weight is a factor that can vary the level of urea in various body fluids of animals. For some authors, MUN concentrations are low in heavy cows (Doska *et al.*, 2012). For others authors, the levels of MUN concentrations are low in the lightdairy cows. Therefore, Doska *et al.* (2012) measured values MUN of 14, 1 mg/dL for Holstein cows, 16, 1 mg/dL for Jersey and 17, 6 mg/dL for Brown Swiss. Those authors hypothesized that the diets in these herds had been formulated according to the milk yield of the dominant breed and subsequently, the Holstein race would have a superior nitrogen utilization efficiency, which could lead to lower MUN concentrations.

However, Johnson and Young (2003) found the higher MUN concentrations for Holsteins (15. 5 mg/dL) and lower concentrations for Jersey (14.1 mg/dL). Thus, when the cows are bigger, they consume more crude protein and increase therefore the production of ammonia. Kauffman and St-Pierre (2001) found no significant difference between MUN concentrations in Holstein and Jersey cows. On the other hand, Oltner *et al.* (1985); Jonker *et al.* (1998); Doska *et al.* (2012) showed that the live weight and breed of the cow are inversely correlated with the MUN level.

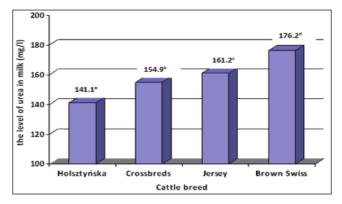


Fig. 4: Average test-day milk urea nitrogen concentrations (mg/L) for different genetic groups (Doska *et al.*, 2012).^{a,b,c} : Means followed by different letters differ (P<0.01) by Tukey test

Logically, if the same amount of urea is produced in cows of different sizes, the urea concentration in the blood and milk will be higher for the small cow.

Feeding and milking frequency

Shabi *et al.* (1998) found that feeding from two to four times a day was accompanied by a decrease in blood urea levels of 4.4 mg/dL. On the other hand, Nilsen *et al.* (2005) found an increase in MUN for the cows milked 3 times per day compared to cows milked twice a day. The unmixed rations composed of fodder, concentrate and mineral supplement separately, predispose cows to the temporary increase of degradable proteins consumption, as well as the volume and duration of ammonia concentration in the rumen and blood. The time of food intake during the day affects diurnal fluctuations in the level of ammonia in the rumen, MUN and in the correlations between urea blood and MUN concentration (Spek, 2013). After ingestion of food by a dairy cow, 4 to 6 hours are necessary to be established a balance between urea concentration in the blood and milk.

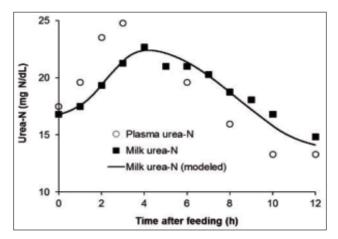


Fig. 5: Urea concentration in cow's milk in consecutive hours after feeding (Spek, 2013).

Thus, a lag of 1.5 to 2 hours was observed between the variation in blood concentration and that in milk (Lefebvre, 1996; Lefebvre *et al.*, 1995). Finally, milk urea sampled during normal milkings represents an easy evaluation of uremia since it is less subject to fluctuations caused by meals (Lefebvre et *al.*, 1995)

Milk yield and milk protein production

The level of milk production is one of the key factors influencing the increase of MUN level (Rzewuska and Strabel, 2013, Sawa *et al.* 2011). The authors Doska *et al.* (2012); Jonker *et al* (1998); Oltner *et al.* (1985) found a positive correlation between milk urea and milk yield. This may lead to the confirmation that highly productive dairy farms may have higher MUN

levels. This correlation observed in highly productive herds explains the energy deficit as the results of the major problem of meeting the energy needs of these animals (Rzewuska and Strabel, 2013). These assertions are similar to those Rajala-Schultz and Saville (2003). Thus, comparing MUN levels throughout lactation for cows producing 8000, 10000, or 12000 kg milk per lactation, Jonker *et al.* (1998) reported that an increase in yield milk of 2000 kg per lactation was associated with an average increase in MUN of 2.6 mg/dL of milk.

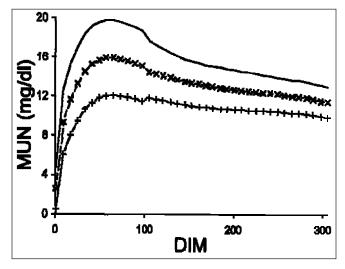


Fig. 6: Predicted milk urea concentration (mg/dL) of cows throughout 305 days 12000 kg (–), 10000 kg (×), and 8000 kg (+) lactations (Jonker *et al.*, 1998)

This positive correlation results from an increase in milk yield linked to the increase of degradable protein percentage in the diet. However, Gustafsson and Carlsson (1993) did not notice a relationship between MUN concentration and cow's production levelwhile Broderickand Claylton (1997) and Roy*et al.* (2003) reported a negative relationship between milk yield and MUN concentrations.

In another study, (Johnson and Young, 2003) suggested that if the milk protein content is high, the level of urea in milk is low. Nelson (1996) had suggested that, when milk protein was ranged from 3.0 to 3.2%, MUN concentration was ranging from 12 to 16 mg/dL and protein

Online ISSN: 2277-3371

degradability fractions and net energy in diet were most likely balanced. In Holstein cows, Johnson and Young (2003) observed the lower MUN concentration (P < 0.0002) when milk protein was >3.2% (vs < 3.0%) for milk yields ranging from 27.3 to 63.6 kg.

Finally, the both significant and non-significant correlations between milk yield, milk protein production, MUN concentrations and other milk components such as casein, lactose and fat were observed in the studies conducted by Butler *et al.* (1996) and Godden *et al.* (2001).

Stage of lactation

The stage of lactation can significantly influence the MUN concentrations and the higher values are usually observed at the peak of lactation than in later lactation (Carlsson *et al.*, 1995; Johnson and Young, 2003). In additional, the urea milk concentration is low during the first month of lactation, increases rapidly for two months and then decreases in the following months (Arunvipas *et al.*, 2003, Canfield *et al.*, 1990, Carlsson *et al.*, 1995).

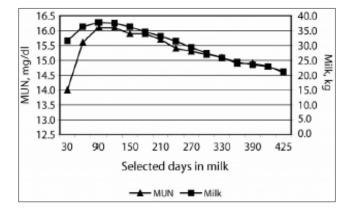


Fig. 7: Relationship between MUN concentrations, milk yield and stage of lactation for dairy cows Holstein (Johnson and Young, 2003)

These results do not agree with those of Ng-Kwai-Hang *et al.* (1985) who reported a rapid decrease in NPN of milk, including urea after calving, followed by a gradual increase until the end of lactation. One of the factors that may

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explain the low level of urea in milk during the first month of lactation is incapacity of dairy cows to ingest a sufficient amount of food at the beginning of lactation accompanied by relatively low consumption of proteins (Carlsson *et al.*, 1995). According to Oltner *et al.* (1985), higher MUN are observed between the 60th and 90th day of lactation. Mucha and Strandberg (2010) reported that the urea content of the milk reaches paroxysm on the 75th day and is maintained until 180th and finally decreases gradually until the end of lactation.

The effect of season, month and herd management

They appear to be a seasonal effect on the level of urea in milk. According to Decruyenaere et al. (1999), milk urea concentrations are high during the summer months and low for the winter months. The results of Arunvipas et al. (2003) indicated that MUN were high in early spring and during the summer and autumn months. In contrast, Doska et al. (2012) showed that MUN were higher in winter, intermediate in spring, and lower in autumn and summer. For Godden et al. (2001), Rajala-Schultz and Saville (2003) and Doska et al. (2012), the milk urea concentrations were high during the warmest months of the year. Furthermore, Carlsson et al. (1993) observed the highest average milk urea when cows were on pasture. Previous studies had found an increase in MUN only one week after entering the pasture and a return to normal after three weeks (Vignon et al., 1978). This return of MUN contents to the normal values might due to a rapid decrease in the nitrogen content of grass in the pasture and the adaptive evolution of the microbial flora of the rumen.

Age and parity

Primiparous cows have generally lower MUN than multiparous because of lower levels of consumption (Arunvipas *et al.*, 2003). Similarly, primiparous cows are still in growth phase and would use amino acids to cover their growth requirements. This would indicate a lower level of NH_3 from deamination in the first lactation cows (Johnson and Young, 2003). In the same logic, Oltner *et al.* (1985) observed that the MUN concentration of primiparous cows was 2.12 mg/dL lower than multiparous cows. These authors also found that weight gain was followed by a decrease in MUN levels of 1.68 mg/dL/100 kg body weight for a growing cows. Although some studies have found lower levels of milk urea for the first lactation cows (Oltner *et al.*, 1985), other studies have reported only very low or even non-significant relationships between parity/age and milk urea levels (Canfield *et al.*, 1990).

However, Jonker *et al.* (1998), and Doska *et al.* (2012) found that the first-lactation cows showed higher (P<0.01) MUN values than those of second lactation and older cows with lactation numbers ≥ 2 . This situation can be related to nitrogen overfeeding because in the majority of dairy farms, the primiparous cows, although less productive, are fed the same diet as adult which are high producing cows (Doska *et al.*, 2012).

The pH of the rumen

The pH and ammonia concentration in the rumen are two major factors that affect the level of ammonia flow in the portal blood (Roy et al., 2011). Scientific researches had shown a strong correlation between pH, CO₂ content, rumen butyric acid, ammonia flux and recycled urea across the rumen wall (Abdoun et al., 2010). The ingestion of a rapidly fermentable carbohydrate diet and the production of volatile fatty acids have an effect on the pH and flow of recycled urea through the rumen wall (Kennedy et al., 1981). So, if the ruminal pH is high, urea recycling is facilitated and the flow of ammonia to the liver is increased (Huntington, 1989), which increases MUN concentration. A ruminal pH below pH 5.5 limits the availability of ammonia and increases the production of volatile fatty acids (Bach et al., 2005). Under these conditions, MUN levels

are low and milk protein production is limited (Abdoun *et al.*, 2010).

Water Intake

Water affects consumption the urea concentration both in the blood plasma and milk (Burgos et al., 2001). Many rations rich in minerals such as Sodium and Potassium induce cows to increase water intake and frequency of urination and consequently to reduce the urea concentration in blood (Bannink et al., 1999). Spek (2013) found that consumption from 69 to 419 g of sodium per day was accompanied by an increase in drinking water of 87.5%, which increased the amount of urine excreted by 49.5 kg/day/cow and a reducing in MUN from 15.2 to 11.8 mg/dL. The urinary reflex that follows the water intake causes a deconcentration of the blood urea which causes a decrease milk urea levels. On the other hand, a limitation to water access exposes dairy cows to dehydration, which increases the concentration of urea in blood and milk. Burgos et al. (2001) found a difference in MUN of 1.58 times higher in cows receiving 50% of their water requirements than their pairs supplied water ad libitum. Those authors suggest that the urea concentration in milk is inversely proportional to the amount of water consumed and the volume of urine produced by the animals.

CONCLUSION

The MUN is considered as an indicator of the nutritional status of dairy herds and gives information on nitrogen efficiency utilization in dairy farming. High urea milk concentrations indicate an excess degradable dietary protein in relation to available energy or a deficiency use of nitrogen in the rumen. In contrast, low MUN concentrations may indicate the very low levels or low digestibility of protein in foods containing a high proportion of fermentable carbohydrates. The variations in MUN concentrations are related to the ratio of proteins/energy, proteins degradability in the rumen and the bacterial capacity to capture excess ammonia in rumen. In any case, changes in MUN concentrations are associated with changes in the diet of the dairy cow. Therefore, maintaining and monitoring the MUN in a dairy herd provides an opportunity to formulate a diet that optimizes the use of nitrogen for dairy production.

REFERENCES

- Abdoun, K., Stumpff, F., Rabbani, I. and Martens, H. 2010. Modulation of urea transport across sheep rumen epithelium in vitro by SCFA and CO₂. Am. J. Physiol., **298**: 190-202.
- Arunvipas, P., Dohoo, I. R., VanLeeuwen, J.A. and Keefe, G.P. 2003 . The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. *Prev. Vet. Med.*, 59(1–2): 83–93.
- Arunvipas P., VanLeeuwen, J.A., Dohoo, I.R. and Keefe, G.P. 2004. Bulk tank milk urea nitrogen: seasonal patterns and relationship to individual cow milk urea nitrogen values. *Can . J. Vet. Res.*, **68**(3): 169 -74.
- Aschemann, M., Lebzien, P., Hüther, L., Südekum, K.H. and Dänicke, S. 2012. Effect of niacin supplementation on rumen fermentation characteristics and nutrient flow at the duodenum in lactating dairy cows fed a diet with a negative rumen nitrogen balance. *Archives of Anim. Nutr.*, **66**(4): 303–318.
- Bach A., Calsamiglia, S. and Stern, M.D. 2005. Nitrogen Metabolism in the *Rumen J. Dairy Sci.*, 88: 9 - 21.
- Bannink, A., Valk, H. and Van Vuuren, A.M. 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. *J. Dairy Sci.*, **82**: 1008-1018.
- Bartley, E.E., Davidovich, A. and Barr, G.W. 1976. Ammonia toxicity in cattle. I. Rumen and blood changes associated with toxicity and treatments methods. *J. Anim. Sci.*, **43**: 835-841.
- Bishonga, C., Robinson, J.J., Mcevoy, T.G., Aitken, R.P. and Findlay, P.A. and Robertson, I. 1994.
 The effects of excess rumen degradable protein in ewes on ovulation rate, fertilization and embryo survival *in vivo* and during *in vitro* culture. *Anim. Prod.*, **58**: 447.

Online ISSN: 2277-3371

\mathcal{N} Munyaneza *et al.*

- Block, E., Dépatie, C., Lefebvre, D. and Petitclerc, D. 1998. L'urée du lait: les sources de variation et les implications. *Symposium sur les Bovins Laitiers*, pp. 77–87.
- Broderick, G.A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.*, **86**(4): 1370–81.
- Broderick, G.A. and Clayton, M.K. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.*, **80**(11): 2964–2971.
- Bruning Fann, C.S. and et Kaneene, J.B. 1993. The effects of nitrate, nitrite and N-nitroso compounds on animal health. *Vet. Hum. Toxicol.*, **35**: 237-253.
- Burgos, S.A., Embertson, N.M. Zhao, Y., Mitloehner, F., DePeters, M. and Fadel, J.G. 2010. Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions. *J. Dairy Sci.*, **93**: 2377 - 2386.
- Burton, H. 1967. Seasonal variation in deposit formation from whole milk on a heated surface. *J. Dairy Res.*, **34**(2): 137–143.
- Butler, W.R. 1998. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle, *J. Dairy Sci.*, **81**(9): 2533–2539.
- Butler, W. R., Calaman, J.J. and Beam, S.W. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J. Anim. Sci.*, **74**(4): 858 – 65.
- Burroughs, W., Nelson, D.K. and Mertens, D.R. 1975. Evaluation of *protein* nutrition by metabolizable protein and urea fermentation potential. *J. Dairy Sci.* **58**(4): 611–619.
- Canfield, R.W., Sniffen, C.J. and Butler, W.R. 1990. Effects of excess *degradable* protein on postpartum reproduction and energy balance in dairy cattle. *J. Dairy Sci.*, **73**(9): 2342–2349.
- Carlsson, J., Bergstrom, J. and Pehrson, B. 1995. Variation with breed, age, season, yield, stage of lactation herd in the concentration of urea in bulk milk and individual cow's milk. *Acta. Vet. Scand.*, **36**: 245–254.
- Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C. and France, J. 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.*, **79**: 240 –246.

- Colmenero, J.J.O. and Broderick, G.A. 2006. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. *J. Dairy Sci.*, **89**(5): 1635–43.
- McDowell, A.K.R. 2009. Seasonal variations in the total nitrogen, non-protein nitrogen and urea nitrogen contents of Friesian and Jersey milk. *J. Dairy Res.*, 39(1): 27.
- Decruyenaere, V., Fabry, J., Van Reusel, A., Bartiaux-Thill, N. 1999. Seasonal variations of milk urea nitrogen and grazing management. *Renc. Rech. Ruminants*, **6**: 315.
- DePeters, E.J. and Ferguson, J.D. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. *J. Dairy Sci.*, **75**: 3192-3209.
- Doska, M.C., Silva, D.F.F., Horst, J.A., Valloto, A.A., Rossi, P. and de Almeida, R. 2012. Sources of variation in milk urea nitrogen in Paraná dairy cows. *Revista Brasileira de Zootecnia*, **41**(3): 692– 697.
- Dufrasne, I., Istasse, L., Lambert, R., Robaye, V. et Hornick, J.L. 2010. Etude des facteurs environnementaux influençant la teneur en urée dans le lait de vache en Wallonie (Belgique). *Biotechnology, Agronomy and Society and Environment*, **14**(1): 59 – 66.
- Enjalbert, F. 2006. Assessment of Nutritional Adequacy in Dairy Cows through diet characteristics and Animal responses. *World Buiatrics Congress*. 180–190.
- Erbersdobler, H.F., Eckart, K., Schwayer, A. and Zucker, H. 1980. Effects of protein and energy intake on milk urea levels. *EAAP Publication*, **27**: 529-533.
- Geerts, N.E., De Brabander, D.L., Vanacker, J.M., De Boever, J.L. and Botterman, S.M. 2004 . Milk urea concentration as affected by complete diet feeding and protein balance in the rumen of dairy cattle. *Livest. Prod. Sci.*, **85**(2–3): 263–273.
- Godden, S.M., Kelton, D.F., Lissemore, K.D., Walton, J.S., Leslie, K.E. and Lumsden, J.H. 2001. Milk Urea Testing as a Tool to Monitor Reproductive Performance in Ontario Dairy Herds. *J. Dairy Sci.*, 84(6): 1397–1406.
- Guliński, P., Salamończyk, E. and Młynek, K. 2016. Improving nitrogen use efficiency of dairy cows in relation to urea in milk - A review. *Anim. Sci. Papers and Reports*, **34**(1): 5–24.

- Gustafsson, A.H. and Carlsson, J. 1993. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. *Livest. Prod. Sci.*, **37**: 91–105.
- Highstreet, A., Robinson, P.H., Robison, J. and Garrett, J.G. 2010. Response of Holstein cows to replacing urea with with a slowly rumen released urea in a diet high in soluble crude protein. *Livest. Sci.*, **129**(1–3): 179–185.
- Hristov, A.N., Grandeen, K.L., Ropp, J.K. and McGuire, M.A. 2004. Effect of sodium laurate on ruminal fermentation and utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. J. Dairy Sci., **87**(6): 1820–31.
- Huntington, G.B. 1989. Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reprod. Nutri. Dev.*, **30**(1): 35-47.
- Huntington, G.B. and Archibeque, S.L., Science, A. and Carolina, N. 2000. Practical aspects of urea and ammonia metabolism in ruminants. *J. Anim. Sci.*, **77**: 1–11.
- Hutjens, M.F. and Barmore, J.A. 1995. Milk urea test gives us another tool. *Hoards Dairyman*, **140**(10): 401.
- Harris, B. Jr. 1995. MUN and BUN values can be valuable management tools. *Feedstuffs*, **67**(42): 14.
- Ide, Y. Ogura, Y. and Yonemura, T. 1966. Observations on the levels of non-protein and urea nitrogen in serum and milk of cows. *Dairy Sci. Abs.*, **28**: 8.
- Johnson, R.G. and Young, A.J. 2003. The Association Between Milk Urea Nitrogen and DHI Production Variables in Western Commercial Dairy Herds. *J. Dairy Sci.*, **86**(9): 3008–3015.
- Jonker, J.S., Kohn, R.A. and Erdman, R.A. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactatig dairy cows. *J. Dairy Sci.*, **81**(10): 2681–2692.
- Jordan, E.R. and Swanson, L.V. 1979. Effect of crude protein on reproductive efficiency, serum total protein, and Albumin in the high producing dairy cow, *J. Dairy Sci.*, **62**(1): 58 63.
- Journet, M., Verite, R. and Vignon, B. 1975. *L'azote non protéique du lait*: facteurs de variation. Le Lait, INRA Editions, **55**(543–544): 212–223.
- Kaim, M., Folman, Y., Neumark, H. and Kaufmann,W. 1983. The effect of protein intake and lactation number on post - partum body weight loss and

reproductive performance of dairy cows. *Anim. Prod.*, **37**: 229 – 235.

- Kauffman, A.J. and St-Pierre, N.R. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *J. Dairy Sci.*, **84**(10): 2284–2294.
- Kebreab E., France J., Mills J.A., Allison, R. and Dijkstra J. 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. J. Anim. Sci., 80: 248-259.
- Kennedy, P.M., Clarke, R.T. and Milligan, L.P. 1981. Influences of dietary sucrose and urea on transfer of endogenous urea to the rumen of sheep and numbers of epithelial bacteria. *Br. J. Nutr.*, **46**: 533-541.
- Kohn, R.A., Kalscheur, K.F. and Russek-Cohen, E. 2002 . Evaluation of Models to Estimate Urinary Nitrogen and Expected Milk Urea Nitrogen. J. Dairy Sci.
- Jonker, J.S., Kohn, R.A. and Erdman, R.A. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci., **81**(10): 2681–2692.
- Kolver, E., Muller, L.D., Varga, G.A. and Cassidy, T.J. 1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *J. Dairy Sci.*, **81**(7): 2017– 2028.
- Kyoung H.K., Oh, Y.G., Choung, J.J. and Chamberlain, D.G. 1999. Effects of varying degrees of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage. *J. Sci. Food and Agri.*, **79**(6): 833–838.
- Lefebvre, D. 1996. L'urée du lait : un outil de plus à votre disposition. Le Producteur de lait québécois, 16(5): 13-15.
- Lefebvre, D., Marchand, D., Léonard, M. Thibault, C., Block, E. and Cannon, T. 1995. Gestion de la perfor- mance du troupeau laitier : des outils à exploiter. 19^{eme}. Symposium sur les bovins laitiers. Saint-Hya- cinthe, Québec. 13-55.
- Meggison, P.A., Mc Meniman N.P. and Armstrong, D.G. 1979. Efficiency of microbial protein synthesis in cattle. *Proc. Nutr. Soc.*, **38**: 146.
- Mucha, S. and Strandberg, E. 2010. Genetic analysis of milk urea nitrogen and relationships with yield and fertility across lactation. *J. Dairy Sci.*, **94**: 5665–5672

Online ISSN: 2277-3371

\mathcal{N} Munyaneza *et al.*

- Nelson, A.J. 1996. Practical application of MUN analyses. *Proc. Am. Assoc. Bov. Prac.*, 85 95.
- Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E. and Monardes, H.G. 1985. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. *J. Dairy Sci.*, **68**(5): 1257– 1262.
- Nickerson, T.A. 1960. Chemical Composition of Milk. J. Dairy Sci., **43**(5): 598–606.
- Nielsen, U. and Clay, J.S. 2005. Milk urea nitrogen concentration: Heritability and genetic correlations with reproductive performance and disease. *J. Dairy Sci.*, **88**: 4434-4440.
- Nocek, J.E. and Russell, J.B. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *J. Dairy Sci.*, **71**(8): 2070–2107.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy of Sciences. National Academy Press, Washington, D.C., USA.
- Nousiainen, J., Shingfield, K.J. and Huhtanen, P. 2004. Evaluation of Milk Urea Nitrogen as a Diagnostic of Protein Feeding. *J. Dairy Sci.*, **87**(2): 386–398.
- Oltner, R., Emanuelson, M. and Wiktorsson, H. 1985. Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. *Livest. Prod. Sci.*, **12**(1): 47–57.
- Oltner, R. and Wiktorsson, H. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livest. Prod. Sci.*, **10**(5): 457–467.
- Parker, D.S., Lomax, M.A., Seal, C.J. and Wilton, J.C. 1995. Metabolic implications of ammonia production in the ruminant. *The Proceedings of the Nutrition Society*, **54**(2): 549 – 563.
- Poncelet, J.L. 1983. L'intoxication ammoniacale ou alcalose de la panse. *La Dépèche technique*, **32**: 39 40.
- Rajala-Schultz, P.J. and Saville, W.J.A. 2003. Sources of Variation in Milk Urea Nitrogen in Ohio Dairy Herds. J. Dairy Sci., 86(5): 1653–1661.
- Roseler, D.K., Ferguson, J.D., Sniffen, C.J. and Herrema, J. 1993. Dietary Protein Degradability Effects on Plasma and Milk Urea Nitrogen and Milk Nonprotein Nitrogen In Holstein Cows. J. Dairy Sci., 76(2): 525–534.

- Rook, J.A.F. 1961. Variations in the milk composition of the milk of the cow. *Dairy Sci. Abst.*, **23**: 251.
- Roy, B., Mehla, R.K. and Sirohi, S.K. 2003. Influence of milk yield, parity, stage of lactation and body weight on urea and protein concentration in milk of murrah buffaloes. *Asian. J. Anim. Sci.*, **16**(9): 1285–1290.
- Roy, B., Brahma, S., Ghosh, P.K., Pankaj and Mandal, G. 2011. Evaluation of Milk Urea concentration as useful indicator for dairy herd management: A Review. Asian J. Vet. Adv., 6(1): 1-19.
- Rzewuska, K. and Strabel, T. 2013. Genetic parameter of milk urea concentration and milk traits in Polish Holstein-Friesian cows. J. Appl. Genetics, 54: 473-482.
- Sairanen, A., Khalili, H., Nousiainen, J.I., Ahvenjärvi, S. and Huhtanen, P. 2005. The effect of concentrate supplementation on nutrient flow to the omasum in dairy cows receiving freshly cut grass. *J. Dairy Sci.*, **88**(4): 1443–1453.
- Salter D.N., Smith R.H. and Hewitt D. 1983. Factors affecting the capture of dietary nitrogen by microorganisms in the forestomachs of the young steer. Experiments with 15N urea. *Br. J. Nutr.*, **50**: 427-435.
- Sawa, A., Bogucki, M. and Krężel-Czopek, S. 2011. Effect of some factors on relationships between milk urea levels and cow fertility. *Archiv. Tierzucht.*, 54: 468-476.
- Schepers, A.J. and Meijer, R.G.M. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. *J. dairy Sci.*, **81**(2): 579 –584.
- Shabi, Z., Arieli, A., Bruckental, I., Aharoni, Y., Zamwel, S., Bor, A. and Hagari, H. 1998. Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digested in the abomasum of dairy cows. J. Dairy Sci., 81: 1991- 2000.
- Shahani, K.M. and Sommer, H.H. 1951. The Protein and Non-Protein Nitrogen Fractions in Milk. The Effect of Heat Treatments and Homogenization. J. Dairy Sci., 34(11): 1035–1041.
- Spek, J.W. 2013. 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. *Anim. Nutr. Group.* Wageningen University, Netherlands, pp. 162.

- Spek, J.W., Dijkstra, J., Van Duinkerken, G. and Bannink, A. 2012. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. *J. Agri. Sci.*, **151**(1–17): 407-423.
- Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci., 75: 345–357.
- Taweel, H.Z., Tas, B.M., Smit, H.J., Elgersma, A., Dijkstra, J. and Tamminga, S. 2006. Grazing behaviour, intake, rumen function and milk production of dairy cows offered Lolium perenne containing different levels of water-soluble carbohydrates. *Livest. Sci.*, **102**(1–2): 33 – 41.
- Van Der Hoek, K.W. 1998. Nitrogen efficiency in global animal production. *Environmental Pollution*, **102**(1): 127–132.
- Wanapat, M., Boonnop, K., Promkot, C. and Cherdthong, A. 2011. Effects of alternative protein sources on rumen microbes and productivity of dairy cows. *Maejo Int. J. Sci. Tech.*, 5(1): 13 – 23.
- Wattiaux, M. A. and Crump, P.M. 2014. Exploring milk urea-N excretion as a nutritional and environmental management tool for the dairy industry. J. Dairy Sci. 97(1): 350.
- Wattiaux, M. A. 2014. Protein Metabolism in Dairy Cows. Materials of the Babcock Institute for International Dairy Research and Development, Wisconsin-Madison, WI 53706, USA.