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**ИСПОЛЬЗОВАНИЕ КОНЦЕНТРАЦИИ МОЧЕВИНЫ В МОЛОКЕ КАК СТРАТЕГИЯ ДЛЯ ПОВЫШЕНИЯ ЭФФЕКТИВНОСТИ ИСПОЛЬЗОВАНИЯ АЗОТА И УМЕНЬШЕНИЯ ПОТЕРЬ АЗОТА В КОММЕРЧЕСКИХ МОЛОЧНЫХ СТАДАХ**

**USING MILK UREA CONCENTRATION AS STRATEGY TO IMPROVE THE EFFICIENCY OF NITROGEN USE AND REDUCING NITROGEN LOSSES IN COMMERCIAL DAIRY HERDS**

Муньянеза Наполеон  
аспирант

[munapol@yahoo.fr](mailto:munapol@yahoo.fr)

*Университет Кади Айяд, 112 Бд. Абдельkrim Аль Хаттаби, 549, 40000, Маракеш, Марокко*

Munyaneza Napoleon  
postgraduate student

[munapol@yahoo.fr](mailto:munapol@yahoo.fr)

*Cadi Ayyad University, 112 Bd. Abdelkrim Al Khattabi, 549, 40000, Marrakech, Morocco*

Нимбона Константин  
аспирант

[cosnim120@yahoo.fr](mailto:cosnim120@yahoo.fr)

*Кубанский государственный аграрный университет, Краснодар, 350044, Россия, Ул. Калинина, 13*

Nimbona Constantin  
postgraduate student

[cosnim120@yahoo.fr](mailto:cosnim120@yahoo.fr)

*Kuban State Agrarian University named after I.T. Trubilin, Krasnodar, 350044, Russia*

Перспективное исследование было проведено на 10 коммерческих молочных стадах в течение одного года по мониторингу мочевины в молоке, определению влияния характеристик рациона на концентрацию и оценке мочевины в молоке, как уменьшить ее в молоке и увеличить выделение азота с мочой. Пробы молока собирали два раза в месяц и анализировали на концентрацию мочевины, используя колориметрическую процедуру. Репрезентативные образцы кормов были также собраны в тот же день сбора молока. В образцах корма были определены концентрации белка, усваиваемого в кишечнике, и чистая энергия для лактации. Расчеты проводили по французской методике, а также определили концентрацию РРБ. Средний диапазон концентраций мочевины в молоке составляет 25,0 - 32,0 мг / дл. Наблюдалась значительная положительная связь ( $p < 0,01$ ) между концентрацией мочевины в молоке и содержанием сырого белка (СБ) в корме. Установлено, что концентрации мочевины в молоке (мг / дл) отрицательно связана ( $p < 0,05$ ) с эффективностью утилизации азота. Была обнаружена тесная положительная корреляция между средними значениями молочной мочевины ( $p < 0,01$ ) и выделения азота с мочой. Отмечена отрицательная связь ( $p < 0,01$ ) между уровнем молочной продуктивности и эффективностью утилизации азота в молочных стадах. Вывод, что количество азота в рационах является наиболее важным фактором питания, влияющим на концентрацию мочевины в молоке. Также коммерческим молочным фермерам может быть полезно отслеживать концентрацию мочевины в молоке, так как это может помочь повысить эффективность использования кормов и минимизировать потери азота в окружающей среде

This prospective study was conducted on 10 commercial dairy herds, over one year on milk urea monitoring, determination of diets characteristics effects on MU concentration and on assessment of MU concentrations as a predictor of N utilization and urinary N excretion. Milk samples were collected twice every month and analyzed for urea concentration using a colorimetric procedure. Representative feed samples were also collected on the same day of milk collection. Feed samples were characterized and their concentrations of protein digestible in the intestine and net energy for lactation were calculated according to the French system as well as PDI requirements. Average of milk urea concentrations range is 25.0 - 32.0 mg/dl. A significant positive association ( $p < 0.01$ ) between MU concentration and CP content was observed. MU concentrations (mg/dl) were found to be negatively associated ( $p < 0.05$ ) with efficiency of nitrogen utilization. A close positive correlation was found between average MU values ( $p < 0.01$ ) and urinary N excretion. A negative association ( $p < 0.01$ ) between level of milk production and efficiency of nitrogen utilization in dairy herds was observed. It is concluded that the amount of nitrogen in diets is the most important nutritional factor influencing MU concentrations and commercial dairy farmers may find it advantageous to monitor milk urea concentration which could help to improve efficiency of feeds use and minimization of N losses to the environment

Ключевые слова: СЫРОЙ БЕЛОК; МОЛОЧНЫЕ КОРОВЫ; УТИЛИЗАЦИЯ АЗОТА; МОЛОЧНАЯ МОЧЕВИНА; ПОТЕРИ АЗОТА

Keywords: CRUDE PROTEIN; DAIRY COWS; NITROGEN UTILIZATION; MILK UREA; NITROGEN LOSSES

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## INTRODUCTION

Interest in the use of milk urea (MU) concentration as a practical indicator of dietary protein status in dairy cows has grown considerably in recent years. In ruminant species, excess protein is converted to ammonia in the rumen which is absorbed into the bloodstream and ultimately detoxified as urea by the liver. Nutritional factors that have major effects on MU concentration are amount of protein in the diets [1], rumen degradable protein and energy/protein ratio [2]. Milk urea is highly associated (0.88 to 0.98) with blood urea and its level represents mainly ( $r = 0.86$ ) N losses from rumen fermentation [3]. Lactating dairy cows eliminate 2.5 to 3% of the urea formed in liver through the milk [4].

The efficiency of N utilization in dairy cows is typically low and highly variable (10% to 40%) compared with the higher efficiency of other production animals [5]. In order to meet the nutritional requirements and sustain milk production, dairy producers often increase nutrients density of diets. However, the efficiency of feed protein is function of the amount of ammonia supply to the rumen and decreases as more crude protein is offered [6]. Although high dietary protein stimulates milk production, overfeeding of protein lead to an increase in urine urea excretion and has been found to be detrimental to reproductive and animal health [7].

In particularly, increasing the level of crude protein in diets increases the amount of nitrogen excreted in cow's urine and then decreases the efficiency of nitrogen utilization. Several studies have shown that excessive milk urea concentration could indicate the insufficient of use of degradable proteins by the micro-organisms present in the rumen, thus reflects excessive nitrogen losses to the environment [8, 9, 10]. The objectives of this study were to (1) examine the

effects of diet composition and nitrogen intake on MU concentrations and (2) assess the potential of MU concentrations as a predictor of urinary N excretion and the efficiency of N utilization under farm conditions.

## **MATERIALS AND METHODS**

### ***Animals and feeding management***

The study was undertaken on Friesian lactating cows from 10 dairy herds located within a semi-arid climatic region of Marrakech in the central part of Morocco, extending between latitudes 30° 50' and 32° 10' North and longitudes 7° 25' and 9° 25' West. During the experimental period, the animals were assigned into groups of 20 to 40 dairy cows. Forage components of diets consisted in corn silage (34.41%), fresh alfalfa (10.75%) and wheat straw (8.85%) completed by concentrate feeds: mixture concentrate feed (29.25%), soybean meal (3.90 %), wheat bran (6.42%) and meal corn (6.42%). Dairy cows were milked twice a day and individually fed after each milking at fixed hours. Farm grown Alfalfa green and wheat straw were offered to all the lactating cows at 08:00 and 14:00h. Maize silage, mixture concentrate feed, soybean meal and vitamin-mineral blend were offered as total mixed rations (TMR) into two equal parts and fed 2hours before each milking in morning( 04:00h) and noon (16:00 h). The dairy cows were milked using a machine and had free access to water throughout the day.

### ***Diet calculation, sampling and milk urea analysis***

Investigative visits were made twice in every month on 10 commercial dairy farms from April 2017 up to march 2018. For each visit the milk sampling and information on composition of diets distributed to dairy cows were collected. Those diets were also characterized for organic matter, crude protein(CP) content, protein digestible in the intestine (PDI) with N or Energy as limiting factor for rumen microbial growth (PDIN and PDIE) and net energy for

lactation (UFL). The dry matter (DM) of forages was determined after drying the samples at 60°C for 48 hours. Diet calculation was based on information relative to feed composition and nutrient value of the feeds and animal requirements using French PDI system with respect to each farm visit. The requirements of the cows in terms of PDI and UFL were estimated following the INRA equations.<sup>11</sup> PDI and UFL balances were then estimated as the difference between allowances and dairy cow requirements, and protein balance in the rumen (OEB = *Onbestendige Eiwit Balans* in the Dutch system) was given by the difference between PDIN and PDIE of rations [12]. To determine the nitrogen intake (Ni), the crude protein (CP) content of the diet ingested was divided by 6.25 ( $Ni, g/d = CP/6.25$ ). The regression equations:  $N \text{ milk } (g/d) = 188 - 0.25*CP$ ;  $NUE (g/d) = -32 + 16.1*MUN$  were developed to predict respectively N in milk and N urinary excreting [6, 13]. The equation ( $ENU = g \text{ N milk } / g \text{ Ni}$ ) was used to estimate efficiency of N utilization [5, 14]. For individual cows, the information on test day milk production, milk fat and protein content, body weight, parity and stage of lactation were collected from farm records. Daily milk productions were adjusted for an identical period of days in lactation in order to compare the milk productions between dairy herds. The milk yield was calculated based on lactation cycle of 305 days. Milk samples were analyzed for urea content using a colorimetric p-dimethylaminobenzaldehyde (DMAB) procedure [15] after little modification. Milk (2 g) was diluted in 500ml of distilled water and deproteinised with 10 ml of Trichloroacetic acid solution, centrifuged at 3000 x g for 30 minutes and filtered. Clear supernatant (5 ml) was mixed with 5 ml of 4-DMAB reagent (1.6 g DMAB + 100 ml ethanol + 10 ml concentrated HCl). The milk urea content was measured at 420 nm absorbance by spectrophotometer UV-3100PC and expressed in milligrams per deciliter (mg/dl) of milk.

### ***Statistical analysis***

Numerical data was analyzed statistically using the Statistical Package for the Social Sciences (SPSS.20). To determine whether effects were significant in

explaining variations in MU concentrations, crude protein (CP) of diet, nitrogen intake (Ni), daily milk production (DMP), efficiency of nitrogen use (ENU) and urinary nitrogen excretion (UNE) the data were analyzed using GLM procedure. The diet crude protein, nitrogen intake, daily milk production, yield milk were taken as sources of variations. The Pearson correlation analysis was performed to investigate the association between different traits. Significant differences were analyzed using the ANOVA test and statistical significance was declared at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### *Effects of CP content of diet on MU concentrations, DMP, ENU and UNE*

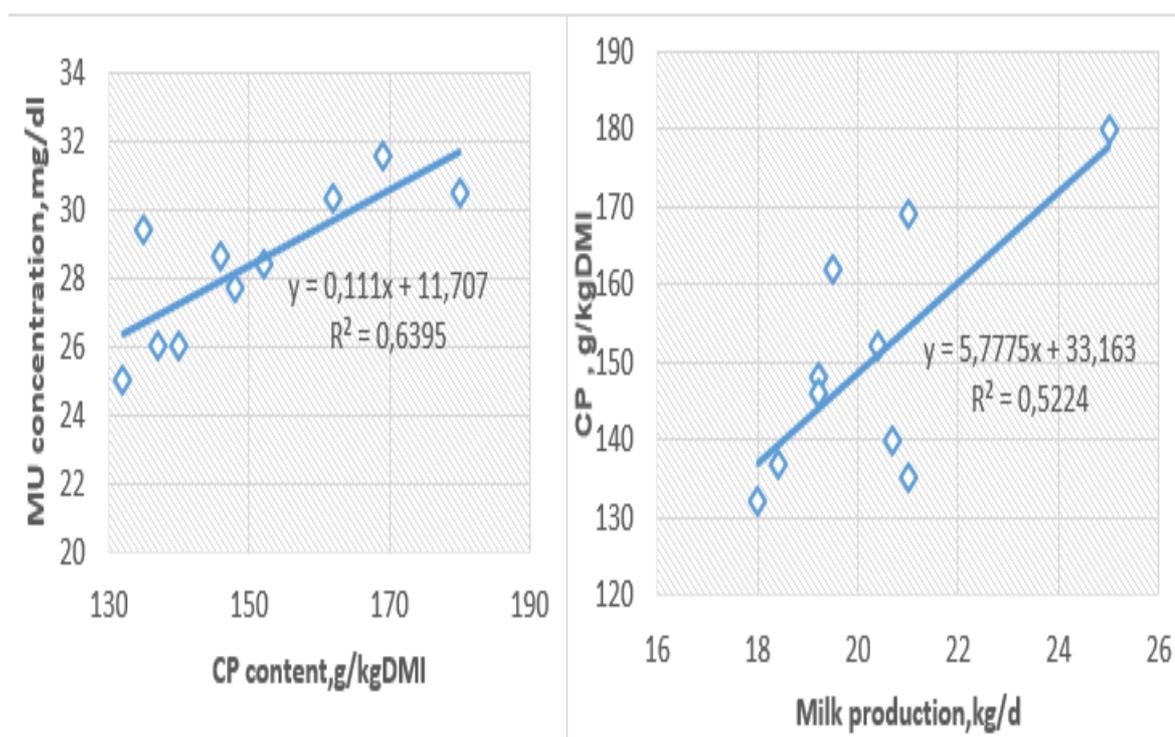
The results on MU concentrations, CP content, DMP, ENU and UNE are presented in table 1. Diets composition have mean values of  $24.5 \pm 2.6$  kg for DM,  $152 \pm 15.8$  for CP content (g/kg DM),  $22.6 \pm 2.7$  for UFL, and  $584 \pm 106$  g/d for Ni and  $-207 \pm 111$  g/d for OEB (Table1).

**Table1: Descriptive analysis of the variables studied.**

Item	Means	SD	Min	Max
Live weight, kg	636	49	532	713
DM intake, kg/d	24.3	2.6	19.9	28.7
CP of diet, g/d	152	15.8	132	180
CP of concentrate mixture, g/d	184	15.7	170	220
N intake, g/d	584	106	419	741
PDI balance				
PDI requirements, g/d	1607	151	1326	1910
PDIN allowances, g/d	2296	428	1706	2985
PDIN balance, g/d	+674	319	+196	+1075
OEB, g/d	-207	111	-325	-68
Aptitude of production				
DMP, kg/d	20.2	2.0	18	25
Milk yield, kg	6185	598	5551	7625
N milk, g/d	150	3.9	143	155
MU concentrations, mg/dl	28.3	2.1	25	32
ENU, %	26.8	5.1	19.3	36.7
UNE, g/d	178	16.2	153	202

SD: standard deviation; DM: dry matter ; MU: milk urea; PDI: protein digestible in the intestine; PDIN: protein digestible in the intestine with nitrogen as limiting factor for rumen microbial growth; OEB (*Onbestendige Eiwit Balans*): rumen protein degradable balance; CP: crude protein; DMP: daily milk production; ENU: efficiency of nitrogen utilization; UNE: urinary of nitrogen excretion.

The offered diets in participating farms were characterized by deficit in rumen degradable protein balance (negative OEB) which signify that the diets had high proportion of rumen degraded protein (RDP) compared to none degraded protein in the rumen of feed ingredients. A negative value of OEB might also indicated inadequate of N intake in lactating cows and therefore, the microbial activity as well as the synthesis of microbial proteins may be impaired [12]. During investigation, the mean of MU concentration was  $28.3 \pm 2.1$  mg/dl. Statistical analysis (ANOVA), did not found a significant variation between MU concentrations in milk samples. This observation may reflect little variation in the quality of the protein fed to the dairy cows. However, the milk urea concentration increased linearly as CP concentrations and N intake increased in the feed (Figure1).



**Figure 1: Relationship among CP content of diets, milk urea concentration and milk production.**

It is noted that high urea in milk indicates that excess protein has been fed to the dairy cow [12] and there high nitrogen losses to environment [5]. In participating dairy farms, the ENU of cows averaged  $26.8 \pm 5.1\%$ . It is noted that ruminants have an overall average ENU (gN in milk /g Ni) of around 25% [10, 14]. A recent study [17] reported that ENU of dairy cows in North America, averages 26.1% of Ni; the corresponding percentages for dairy cows in Northern Europe is 27.4%. These regional differences might be explained in part by the difference in Ni. Other study showed that from 20 to 35% of ingested nitrogen is excreted as protein in milk and that up to 50% of consumed nitrogen is found in the urine [12]. As ruminant, the study showed that the conversion of feed N into milk protein (which explain level of ENU) of dairy cow Holstein in semi-arid conditions of central Morocco is low. This low ENU has implications not only for production performance and profitability but also for the emission of contaminants to the environment. In particularly, the findings of this study showed that dairy cow Holstein in semi-arid area of central Morocco, excretes N ( $178 \pm 16.2\text{g /d}$ ) in urinary urea form and it secretes N ( $150 \pm 3.9\text{g/d}$ ) as milk protein. Recent study conducted in North America reported that the lactating dairy cow excretes as much urinary urea-N ( $168\text{ g/d}$ ) than it secretes N ( $166\text{ g/d}$ ) as milk protein [18]. Generally, increasing the percentage of dietary crude protein increases N intake and urinary urea-N excretion and decreases ENU. Through an extensive literature survey [19], the commonly accepted “optimal” MU concentrations of 21.7 to 25.7 mg/dl which reflects high ENU but the milk protein production can be at maximum for any MU concentrations values ranging from 21.7 to 34.7 mg/dl [18, 20].

***Relationship of milk urea concentrations, CP content of diets, milk production, ENU and UNE.***

The findings on associations among MU concentrations, CP content of diets, milk production, ENU and UNE are presented in Table 2. During investigation, MU concentrations was found to be significantly associated with CP content of diet ( $P < 0.00$ ). A study also reported marginally closer relationships between MU concentrations and dietary CP content [3]. A close positive correlation was found between MU concentration ( $p < 0.05$ ) and CP content of mixture concentrate in agreement with the results obtained by Dhali et al. [15]. A negative correlation was found ( $p < 0.05$ ) between MU concentrations and ENU in agreement with the results obtained by Nousiainen et al. [21]. Then again, CP content of mixture concentrate was found to affect negatively the ENU in dairy farms and increased significantly the nitrogen losses in environment (Figure2).

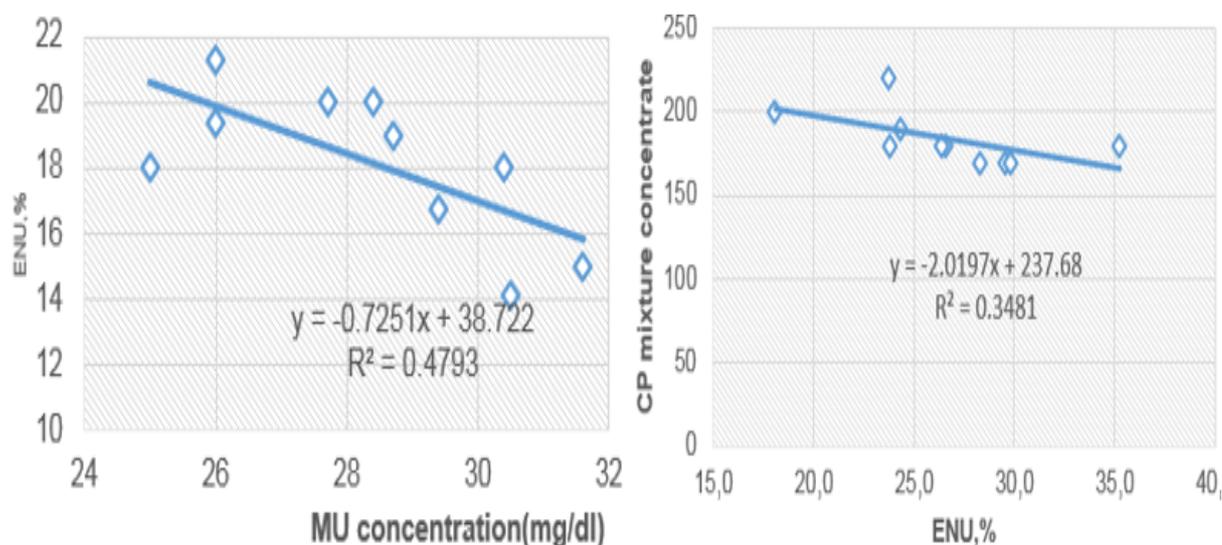
**Table 2: Relationship of milk urea concentration and others factors affecting efficiency of nitrogen utilization.**

Particulars	Correlation coefficient (r)	
	MU concentration	ENU,%
Number of cows	0.761*	-0.703*
CP content of diet (g/d)	0.738**	-0.646*
CP of concentrate mixture(g/d)	0.775**	-0.626
N intake(g/d)	0.791**	-0.821**
DMP (kg/d)	0.538	-0.630
Milk yield(kg)	0.765**	-0.825**
N milk(g/d)	0.901	0.252
ENU (%)	-0.669*	1
UNE (g/d)	0.988**	-0.692*
MU concentrations	1	-0.669*

\* Indicates the r value is significant at  $p < 0.05$ .

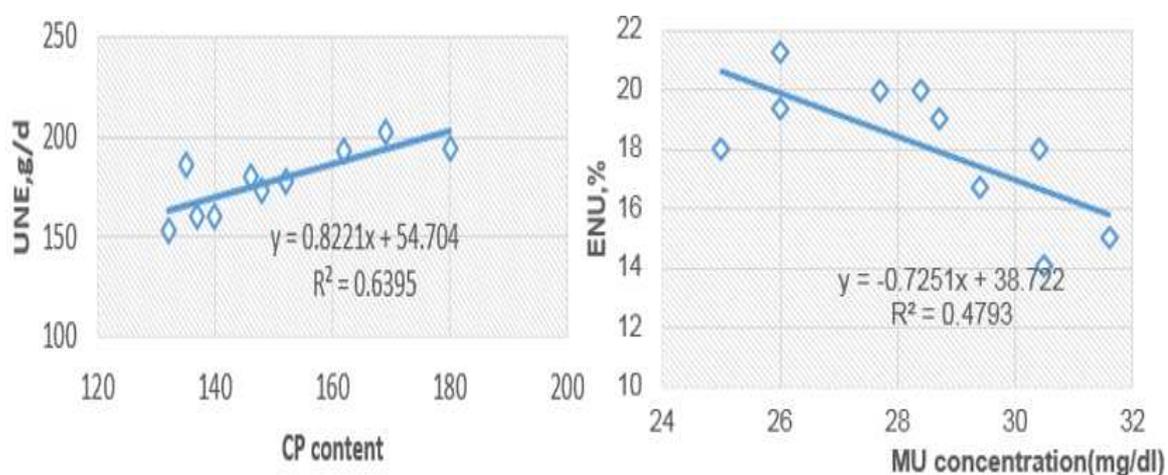
\*\* Indicates the r value is significant at  $p < 0.01$ .

**Abbreviations: See table1**



**Figure 2: Relationship among CP content mixture, ENU and UNE**

A negative relationship observed among the ENU and many factors such as urea in milk, CP content of diet, CP mixture concentrate, DMP and UNE. This observation might explain that the efficiency of dietary protein for milk production decreases as more protein was offered. During investigation, the UNE was significantly increased when the CP content of diet was high and the ENU tended to decrease when the MU concentrations increased (Figure 3).



**Figure 3: Association between CP mixture concentrate, MU concentration and ENU.**

Study of Ciszuk and Gebregziabher confirmed the same observation [22]. It is noted that increase CP mixture concentrate in diets based in lower CP forage lead to reducing in converting of N dietary to milk protein [23] and

increase the N excretion in urine and milk [16]. Subsequently, it was reported that a reduction in dietary CP of 18% to 16.2% (dietary dry matter basis) allowed reducing urinary urea-N from 178 g/d to 134 g/d which could represented 24.7% of reduction [18]. Coefficients of correlation between factors showed in Table 2 confirm that the amount of N intake has an effect on MU concentrations, which is evidence of that overfeeding proteins contribute to reduce the ENU of dairy cows and to N pollution of the environment.

After all, the excessive use of protein supplements in modern dairy farm could constitute a feeding strategy to increase urea in milk and losses of nitrogen in the form of urinary urea nitrogen and may translate into additional costs [24]. Therefore, commercial dairy farmers may find it advantageous to monitor MU concentration, which could help to improve efficiency of nitrogen utilization and decreases N losses in dairy farms.

## CONCLUSIONS

The urea concentration of cow's milk can be utilized for a finer tuning of protein feeding, in order to improve milk N efficiency and reduce urinary N excretion. The main factor influencing MU concentrations is the amount of crude protein content in diet offered to dairy cows. The impacts of changing strategies of feeding on commercial dairy farms to improve the efficiency of nitrogen use and reducing N excretion could be monitored through changes in milk urea concentrations.

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