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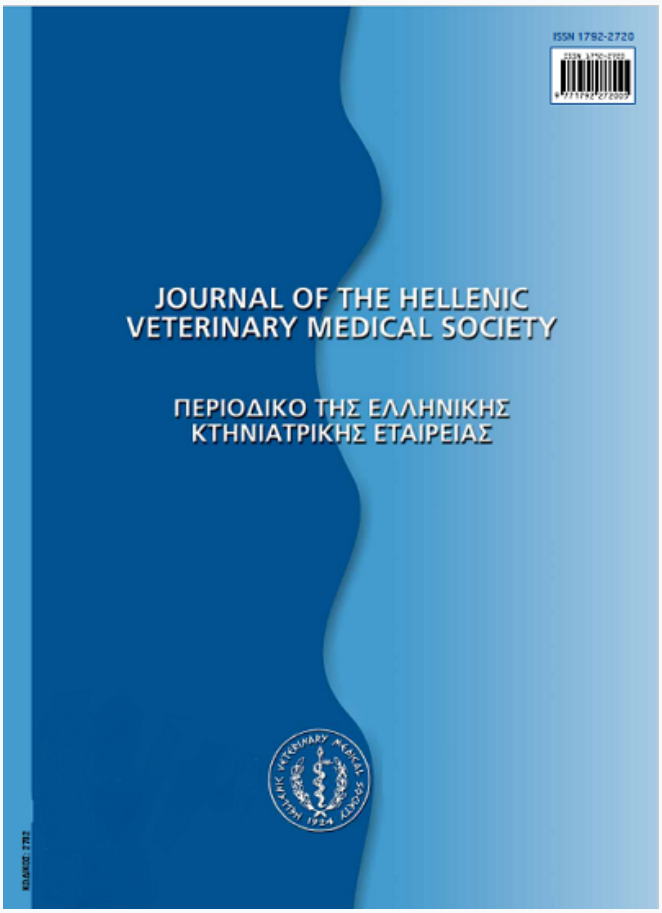

Investigation of milk urea nitrogen concentration and factors affecting its variation in Greek Holstein herds

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Research article
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Investigation of milk urea nitrogen concentration and factors affecting its variation in Greek Holstein herds

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Διερεύνηση της συγκέντρωσης ουρεϊκού αζώτου γάλατοςκαι των παραγόντων που επηρεάζουν τη διακύμανσή του σε ελληνικές εκτροφές αγελάδων φυλής Holstein

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ABSTRACT. Milk urea nitrogen (MUN) is an important tool in dairy cow nutrition, as it reflects the amount of nitrogen in the diet which is not used for production. The objective of this study was to evaluate MUN values in Greek dairy herds, for the first time, and to investigate the source of its possible variation. For this purpose, a dataset of 23,266 milk records from 24 Holstein herds in the region of Thessaly (Greece) was used. Descriptive statistics, analysis of variance and a multiple regression model were used for statistical analysis. Significant differences were observed among farms ($P<0.05$). Mean MUN concentration was 15.54 mg/dL. More than 90% of the measurements were above the upper limit of reference herd target-values. In contrast with previous observations, lower MUN values ($P<0.05$) were observed during the summer and autumn. A positive relationship between milk yield and MUN was observed, but only up to MUN values of 16 mg/dL. Milk fat content and fat/protein ratio were negatively related to MUN, while cows with higher protein content had lower MUN values ($P<0.05$). Most milk traits and sampling month explained only 25.8% of the variation in

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MUN concentration (P<0.05). In conclusion, MUN values in Greek dairy farms were greater than target-values suggested for most herds, indicating systematic nutritional errors that could affect health and reproductive performance of dairy cows.

Key words**:** milk urea nitrogen, milk yield, milk composition, dairy cows, Greek herds

ΠΕΡΙΛΗΨΗ. Η μέτρηση της συγκέντρωσης του ουρεϊκού αζώτου (MUN) στο γάλα αποτελεί ένα χρήσιμο εργαλείο στη διατροφική διαχείριση των εκτροφών γαλακτοπαραγωγών αγελάδων, καθώς είναι ενδεικτικό της ποσότητας του αζώτου της τροφής που δεν αξιοποιείται. Σκοπός της παρούσας εργασίας ήταν να μελετηθούν, για πρώτη φορά, οι συγκεντρώσεις του MUN στο γάλα ελληνικών εκτροφών και να ερευνηθούν οι παράγοντες που επηρεάζουν τη διακύμανσή του. Για το λόγο αυτό, χρησιμοποιήθηκαν 23.266 ατομικές γαλακτομετρήσεις από 24 εκτροφές αγελάδων φυλής Holstein στην περιοχή της Θεσσαλίας. Για τη στατιστική επεξεργασία χρησιμοποιήθηκαν μέθοδοι περιγραφικής στατιστικής, ανάλυσης διακύμανσης και πολλαπλής παλινδρόμησης. Παρατηρήθηκαν σημαντικές διαφορές μεταξύ των εκτροφών (P<0,05). Η μέση τιμή του MUN στο σύνολο των εκτροφών ήταν 15,54 mg/dL. Περισσότερες από το 90% των μετρήσεων ήταν υψηλότερες από τις τιμές-στόχο που προτείνονται για τις περισσότερες εκτροφές. Σε αντίθεση με τα περισσότερα βιβλιογραφικά δεδομένα, οι τιμές MUN ήταν χαμηλότερες (P<0,05) τους καλοκαιρινούς και φθινοπωρινούς μήνες. Η συγκέντρωση του MUN αυξανόταν όσο αυξανόταν το ύψος της γαλακτοπαραγωγής, αλλά μόνο μέχρι την τιμή MUN των 16 mg/dL. Το ποσοστό του λίπους στο γάλα και ο λόγος λίπους/πρωτεϊνών σχετιζόταν αρνητικά με τη συγκέντρωση του MUN, ενώ αγελάδες με υψηλή περιεκτικότητα πρωτεϊνών στο γάλα είχαν χαμηλότερο MUN (P<0,05). Το ύψος της γαλακτοπαραγωγής, τα συστατικά του γάλατος και ο μήνας της μέτρησης ευθύνονταν μόνο για το 25,8% της διακύμανσης των τιμών του MUN (P<0,05). Συμπερασματικά, η συγκέντρωση του MUN στο γάλα στις ελληνικές εκτροφές είναι μεγαλύτερη από τις τιμές-στόχο που προτείνονται διεθνώς, γεγονός που δηλώνει συστηματικά διατροφικά σφάλματα με πιθανές επιπτώσεις στην υγεία και τη γονιμότητα των αγελάδων.

Λέξεις ευρετηρίασης: ουρία στο γάλα, γαλακτοπαραγωγή, αγελάδες Holstein, ελληνικές εκτροφές

INTRODUCTION

Modern dairy cows produce much more milk than in the past but, at the same time, their fertility is significantly decreased (Lucy, 2001). Besides genetics, management issues including increased farm size, heat stress and nutrition, present the greatest challenges. Among others, the quality and quantity of crude protein (CP) offered to dairy cows in order to meet the increased demands of high milk production, represent important dietary factors affecting fertility (Staples and Thatcher, 2001).

Feeding excessive amounts of CP and/or ruminally degradable CP is positively correlated with increased production of ammonia (NH_3) in the rumen. Excess NH_3 is absorbed through the ruminal mucosa, enters the portal circulation and is transported to the liver where it is metabolized to urea (Staples et al., 1992). This conversion is necessary because NH_3 is very toxic to several tissues. Blood urea is partly recycled in the rumen, excreted via urine and secreted in milk. Blood urea is transported by passive diffusion to the mammary gland (Clark et al., 1978), resulting in an equilibrium between the values of urea in blood and those in milk (Gustafsson and Palmquist, 1993). Levels of blood urea nitrogen (BUN) are highly correlated to those of milk urea nitrogen (MUN) (R^2 = 0.842) (Broderick and Clayton, 1997).

High blood NH_3 and urea levels have adverse effects on certain reproductive processes (Butler, 2000), which cause significant economic losses to dairy farmers (Rhoads et al., 2008). According to Ferguson et al. (1988), cows with BUN >20 mg/dL are three times less likely to be diagnosed pregnant compared to cows with BUN ≤20 mg/dL. Similarly, Melendez et al. (2000) reported that cows with elevated MUN

(17-25 mg/dL) were three times less likely to become pregnant than cows with lower MUN concentration (6-16 mg/dL). The various causes of the negative impact of high blood NH_3 and urea concentrations on reproduction are: 1) the additional energy costs for the excretion of excess nitrogen (N) in early lactation (Staples et al., 1993; Butler, 1998; Leroy et al., 2008), 2) the direct toxic effect of NH_3 and urea on gametes, follicles and oviduct (Visek, 1984; Ferguson et al., 1988; Staples et al., 1993; Moore and Varga, 1996), 3) the reduction of pH in the uterus lumen (Elrod and Butler, 1993), 4) the alteration in potassium, magnesium, phosphorus and zinc ion concentrations and the increased urea concentration in uterine secretions (Jordan et al., 1983) and 5) the reduction in plasma progesterone levels (Jordan and Swanson, 1979; Sonderman and Larson, 1989).

Collecting a milk sample for urea or MUN measurement is practically much easier, faster and cheaper than collecting a blood sample for BUN measurement to assess a herd feeding program (Baker and Ferguson, 1993). Some laboratories report milk urea values instead of MUN and, in order to convert urea values to MUN, they must be divided by 2.14, since the urea molecule consists of 46.65% N. Target values for mean MUN concentration range between 8.5 and 11.5 mg/dL for most dairy herds (Kohn et al., 2002). However, Aguilar et al. (2012) suggest that common target values should not be used for all farms without taking into account the normal MUN variation among herds.

For the correct interpretation of herd MUN values, the factors affecting their variation must be consid-

Table 1. Descriptive statistics for milk yield and milk traits

Variable	Mean	SD	Minimum	Maximum
Milk yield (L/d)	27.31	9.75	3.50	69.90
FCMY[1] (L/d)	26.16	8.67	2.67	86.29
Fat %	3.84	.91	.40	12.99
Protein %	3.49	.47	2.14	9.82
FPR[2]	1.10	.24	.14	4.48
MUN[3] (mg/dL)	15.45	3.08	.99	36.80
Lactose %	4.83	.30	1.83	5.67
SNF[4] %	8.96	.52	5.56	14.74
SCC[5] (x1000/mL)	419.95	1020.59	.00	24704.00
SCS[6]	2.14	.62	.00	4.39
Fat yield (g/d)	1015.76	353.09	76.76	4565.70
Protein yield (g/d)	931.60	292.00	120.40	2862.96
Lactose yield (g/d)	1327.65	493.65	89.30	3329.48

1 FCMY: Fat-Corrected Milk Yield (Gaines, 1928)
2 FPR: Fat/Protein Ratio
3 MUN: Milk Urea Nitrogen
4 SNF: Solids Non-Fat
5 SCC: Somatic Cell Count
6SCS: Somatic Cell Score

ered. The main dietary factors known to affect MUN concentration are the CP content, the protein - energy balance of the diet, the degree of protein degradation in the rumen, the amount of rumen NH_3 that exceeds microbial requirements and the total intake of protein and energy in relation to their requirements (Carlsson et al., 1995; Hof et al., 1997).

High milk yield has been associated with high MUN concentration, (Oltner et al., 1985; Carlsson et al., 1995), as milk yield is directly related to the protein/energy ratio of the diet under controlled dietary conditions (Oltner et al., 1985). The relationship between MUN and various milk components (such as fat, protein and lactose content, fat/protein ratio and somatic cell counts) has been investigated in several studies, with controversial results (Broderick and Clayton, 1997; Godden et al., 2001a; Johnson and Young, 2003; Hojman et al., 2004; 2005; Doska et al., 2012; Fatehi et al., 2012; Meyer et al., 2012). Moreover, MUN concentration fluctuates during the year, with higher values usually occurring during the warm months (Carlsson et al., 1995; Godden et al., 2001a; Arunvipas et al., 2003; Hojman et al., 2004; Wattiaux et al., 2005; Bastin et al., 2009; Fatehi et al., 2012).

MUN is widely used in Europe and North America as a tool for monitoring diets and limiting the negative impact of excess N intake on cow's reproduction and the environment. In Greece, milk urea measurements are not routinely available, despite the fact that they are included in the monthly production records provided from the Holstein Association of Greece to its members (http://holstein.gr/index.php?q=node/13).

Table 2. Milk urea nitrogen (MUN) concentration (mg/dL) by milk traits classes

Class	Milk yield			FCMY[1]			Fat			Protein		
	n	(L)	Mean	n	(L)	Mean	n	(%)	Mean	n	(%)	Mean
1	5204	<20	15.32^a	5756	<20	15.52^a	8228	<3.5	15.74^a	3010	<3.0	15.51^a
2	9620	20≤..≤30	15.36^a	10022	20≤..≤30	15.38^b	10462	3.5≤..≤4.5	15.49^b	3591	3.0≤..≤3.2	15.49^a
3	8442	>30	15.63^b	7488	>30	15.49^a	4576	>4.5	14.83^c	4015	3.2<..≤3.4	15.62^a
4										4042	3.4<..≤3.6	15.56^a
5										3301	3.6<..≤3.8	15.46^a
6										5307	>3.8	15.17^b

Class	FPR[2]			Lactose			SNF[3]			SCS[4]		
	n	(ratio)	Mean	n	(%)	Mean	n	(%)	Mean	n	(logSCC)	Mean
1	6666	<1	15.75^a	3661	<4.6	15.44^a	3896	<8.5	15.64^a	10092	<2.0	15.37^a
2	10160	1≤..≤1.2	15.49^b	16465	4.6≤..≤5.1	15.30^b	12300	8.5≤..≤9.2	15.52^a	8003	2.0≤..≤2.6	15.39^a
3	6440	>1.2	15.09^c	3140	>5.1	16.25^c	7070	>9.2	15.23^b	5115	>2.6	15.70^b

Class	Fat yield			Protein yield			Lactose yield		
	n	(g)	Mean	n	(g)	Mean	n	(g)	Mean
1	4344	<700	15.63^a	4064	<650	15.38^a	6247	<1000	15.32^a
2	12303	700≤..≤1200	15.40^b	9760	650≤..≤1000	15.37^a	8807	1000≤..≤1500	15.34^a
3	6619	>1200	15.43^b	9442	>1000	15.56^b	8212	>1500	15.67^b

Means with different superscripts within the same column differ ($P \leq 0.05$).

1FCMY: Fat-Corrected Milk Yield (Gaines, 1928)

2FPR: Fat/Protein Ratio

3SNF: Solids Non-Fat

4SCS: Somatic Cell Score (log-transformation of SCC)

The aim of this study was to: a) evaluate for the first time MUN concentrations on Greek dairy farms and compare them with reference herd target-values, and b) investigate the effect of some non-nutritional factors (month or season, milk yield and components) on MUN concentration variance in Greek Holstein herds.

MATERIALS AND METHODS

A dataset, consisted of 23,266 monthly milk records from 3,715 Holstein cows kept in 24 commercial dairy herds from the Thessaly region (Central Greece), was used in the study. Samples were collected from January 2009 until March 2010, and analyzed with Fourier Transform Infrared Spectroscopy (FTIR) analytical technology using a Milkoscan analyzer, by the local branch of the Holstein Association of Greece. There were no records for August 2009. Each record included the following: a) cow number, b) date, c) milk yield (MY) (L), d) fat (F) (%), c) protein (P) (%), d) lactose (L) (%), e) solids-non-fat (SNF) (%), f) somatic cell count (SCC) (x1000 SC/mL) and g) urea (mg/dL). Milk protein values represent the crude protein content. Urea values were converted to MUN using the equation: MUN (mg/dL) = Urea (mg/dL) / 2.14. Furthermore, fat, protein and lactose yield (g) (FY, PY and LY, respectively), fat/protein ratio (FPR), as well as the 4% fat-corrected-milk-yield [FCMY – according to Gaines' formula (Gaines, 1928)] were calculated. Somatic cell count was log-transformed to somatic cell score (SCS). Mean monthly temperature and relative humidity records of the region, where the herds were located, were obtained from the Hellenic National Meteorological Service. The mean monthly Temperature-Humidity Index (THI) was calculated according to the following formula (NRC, 1971): *(1.8*T+32) – [(0.55-0.0055*RH)*(1.8*T-26)]*; where T: mean monthly temperature (in degrees Celsius) and RH: mean monthly relative humidity (in %).

Most milk traits were grouped in three classes (1: low, 2: medium, 3: high) as follows: a) MY and FCMY: <20 L, 20-30 L and >30 L, respectively, b) F: <3.5%, 3.5-4.5% and >4.5%, c) L: <4.6%, 4.6-5.1% and >5.1%, d) SNF: <8.5%, 8.5-9.2% and >9.2%, e) FY: <700 g, 700-1,200 g and >1,200 g, f) PY: <650 g, 650-1,000 g and >1,000 g, g) LY: <1,000 g, 1,000-1,500 g and >1,500 g, h) FPR: <1.0, 1.0-1.2 and >1.2, and i) SCS: <2.0, 2.0-2.6 and >2.6. Regarding P, and in order to accurately detect differences, 6 classes (<3.0%, 3.0-3.2%, >3.2-3.4%, >3.4-3.6%, >3.6-3.8% and >3.8%, respectively) were formed. To further explore the association between MUN and MY, MUN was grouped in 11 classes, in increments of 2 mg/dL, with those of ≥24 mg/dL as the upper category, according to Johnson and Young (2003). Moreover, in order to explore the association between MUN and the combination of P with MY, 6 new MY classes were created, in increments of 9.1 L/d up to 63.6 L, according to Johnson and Young (2003).

The homogeneity of variances was assessed by Levene's test. Normal distribution of data was confirmed by normality plots. Multiple comparisons were made using Bonferroni and Tukey's procedures. Analysis of variance (ANOVA) was used to determine the association between MUN and herd, the above milk traits, month and season (Spring: March-April-May, Summer: June-July, Autumn: September-October-November, Winter: December-January-February); ANOVA was also used to determine the association between month and season with MY, F and P.

Multivariate linear regression was used to determine the influence of milk traits, mean monthly temperature, relative humidity and THI (continuous scale), and month or season (discrete scale) on MUN and to examine the predictive value of a model with non-nutritional factors. Variables were included in the regression equation following the "forward" selection method.

The statistical analysis was performed using the IBM SPSS Statistics V.22 software package. The significance level was set at $P \leq 0.05$.

RESULTS

Descriptive statistics are presented in Table 1. Mean (±SD) MY and FCMY were 27.31±9.75 L and 26.16±8.67 L, respectively. Mean F, P, SCC and SCS were 3.84%, 3.49%, 419.950/mL and 2.14, respectively.

All herds had mean MUN values above the suggested target range of 8.5-11.5 mg/dL, in all monthly tests. There were significant differences among herds ($P<0.05$), with a herd-level minimum at 13.58 mg/dL

and a maximum at 16.56 mg/dL.

Mean cow-level MUN (test-day records) was 15.45 mg/dL. The coefficient of variation (CV) of cow-level MUN was 19.91%. Cow-level MUN values <8.5 mg/dL were observed in only 0.9% of the records (n = 200), 8.5-11.5 mg/dL in 6.7% (n = 1,551) and >11.5 mg/dL in 92.4% of the records (n = 21,515); 50% of the MUN records were ≥15.2 mg/dL and 17.06% ≥18 mg/dL. Among cows of the same farm the minimum CV was 10.35% and the maximum 36.24%.

Cows with a daily MY and FCMY >30 L had greater MUN values (P<0.001); however, MY did not change when MUN values were above 16 mg/dL. As F and FPR increased, MUN concentration decreased significantly (P<0.001). Cows with P >3.8% had lower MUN values (P<0.001). When MY and P classes were combined, cows with higher P (>3.80%) had statistically significant (P<0.05) lower MUN values in some MY classes and a similar tendency in others. However, cows with high daily PY (>1,000 g) had greater MUN values (P<0.01). Higher L and LY were associated with greater MUN values (P<0.001), while higher SNF was associated with lower ones (P<0.001). In addition, a SCS of >2.6 was associated with greater MUN values (P<0.001). Despite statistical significance, all the above differences were numerically small (Tables 2 & 3).

Milk yield (MY), MUN, F and P by season and month of the year are presented in Table 4. There were significant differences among test months (P<0.05). Cow-level MUN was at its lowest in December (13.39 mg/dL) and at its maximum in February (17.06 mg/dL). MUN values were higher in winter (15.80 mg/dL) and spring (15.89 mg/dL), decreased in summer (15.11 mg/dL) and reached a minimum in autumn (14.32 mg/dL) (P<0.001). Milk yield, also, decreased from spring (27.83 L) to summer (26.81), reached a minimum in autumn (25.94 L) and recovered in winter (27.78 L) (P<0.001). Additionally, milk protein percentage was at its lowest in summer (3.36%) and increased in autumn and winter (3.55%, both seasons) (P<0.001).

Test month, F, SCS, SNF, P, L, FY, MY and LY were the explanatory variables entered in the regression model that best predicted the MUN value. The adjusted correlation coefficient (R^2) of this model was 0.258.

DISCUSSION

Mean MUN in our study was 15.45±3.08 mg/dL. Similar concentrations have been reported by Johnson and Young (2003) and Hojman et al. (2005) while Aguilar et al. (2012) and Fatehi et al. (2012) reported higher MUN values. In most cases, however, lower MUN values have been reported (Arunvipas et al., 2003; Nousiainen et al., 2004; Hojman et al., 2004; Wattiaux et al., 2005; Bastin et al., 2009; Doska et al., 2012). In general, increased MY is associated with increased MUN values. This was the case in our study, as well. However, mean MY in our study was similar (Arunvipas et al., 2003; Nousiainen et al., 2004) or lower (Godden et al., 2001b; Johnson and Young, 2003; Hojman et al., 2004; Aguilar et al., 2012; Fatehi et al., 2012; Doska et al., 2012) than what has been reported in other MUN studies. Other parameters must also be considered.

Only Arunvipas et al. (2003) reported a CV in MUN values similar to ours. Greater variance (22.7-42%) was found by Johnson and Young (2003), Nousiainen et al. (2004), Bastin et al. (2009), Aguilar et al. (2012), Fatehi et al. (2012) and Doska et al. (2012).

In agreement with most previous studies and in contrast with Hojman et al. (2004; 2005), higher F and P values in the present study were associated with lower MUN values. Despite the similar trend though, higher overall mean F and P values, compared to other studies, were accompanied by overall higher MUN values. The negative relationship between F and MUN has been reported in many studies (Broderick and Clayton, 1997; Godden et al., 2001a; Johnson and Young, 2003; Doska al., 2012; Fatehi et al., 2012). In our study, this relationship was observed up to the MUN class of 18 mg/dL. Milk F tends to increase as MY declines. This inverse relationship could be due to the fact that MUN is positively correlated with MY. In addition, F increases by administering forages that enhance the production of acetic acid in the rumen. Rations rich in ruminally degradable fiber favor the growth of cellulolytic bacteria, reducing thus the concentration of NH_3 in the rumen, which is the preferred source of N for these microbes, and, eventually, decrease the amount of excess N to be converted to urea

Table 3. Milk yield by milk urea nitrogen (MUN) classes

MUN Class (mg/dL)	n	Milk Yield (L)	
		Mean	SD
≤6.0	32	21.37[a]	8.53
6.01-8	99	21.57[a]	9.33
8.01-10	471	23.74[ab]	8.48
10.01-12	1883	25.53[bc]	9.17
12.01-14	5020	27.45[c]	9.68
14.01-16	6762	28.01[c]	9.79
16.01-18	5034	27.54[c]	9.76
18.01-20	2242	26.97[c]	9.87
20.01-22	929	26.61[bc]	9.75
22.01-24	509	27.88[c]	10.48
>24	285	28.26[c]	9.94

Means with different superscripts within the same column differ (P ≤ 0.05).

(Hristov and Ropp, 2003).

Similarly to F, P decreased as MUN increased up to the class of 18 mg/dL. However, only cows with P >3.8% and PY >1000 g had significantly lower MUN. Arunvipas et al. (2003) found that the correlation between MUN and P was -0.212. Johnson and Young (2003) explained this negative relationship as a result of the connection of low MUN levels with better N utilization efficiency for milk protein synthesis. Furthermore, Fatehi et al. (2012) reported that dairy cows of the same MY class had significantly lower MUN as P increased. We found a similar tendency in our study, but, only for some MY classes.

In agreement with Fatehi et al. (2012), a negative relationship between MUN and SNF was found in our study, but, again, only up to the MUN class of 18 mg/dL. After that, SNF percentage increased. Moreover, Hojman et al. (2004; 2005), Johnson and Young (2003) and Arunvipas et al. (2004) reported a negative correlation between MUN and SCC. Licata et al. (1985) reported that quarters with a positive California mastitis test had 2.7 mg/dL less MUN than negative ones. In contrast to those findings, we found that cows with a SCS more than 2.6 had significantly higher MUN (P<0.001). A possible explanation for the positive correlation between MUN and SCS is that the decrease in MY in cows with subclinical mastitis is not followed by a simultaneous reduction in dry matter and CP intake, resulting to a larger amount of excess N that has to be eliminated as MUN.

MUN values follow a seasonal fluctuation through the year, with higher concentrations usually occurring in summer months (Carlsson et al., 1995; Godden et al., 2001b; Arunvipas et al., 2003; Hojman et al., 2004; Wattiaux et al., 2005; Bastin et al., 2009; Fatehi et al., 2012; Rzewuska and Strabel, 2013b). It should be noted that heat stress increases the catabolism of body protein by intense muscle breakdown (Schneider et al., 1988; Kamiya et al., 2006). The increase in blood urea levels represents a physiological alteration during a heat stress period (Wheelock et al., 2010). The seasonal variation in MUN is due to a cumulative parameter representing an overall environmental impact (e.g. temperature, humidity and photoperiod), changes in dietary management (e.g. spring grazing), the level of MY and the distribution of calvings through the year (Wattiaux et al., 2005). Rajala-Schultz and Saville (2003) observed only small differences among seasons in high-producing dairy farms (>10,433 kg of milk/cow/year), where cows were kept indoors all year, with lower MUN recorded during the summer months. In addition, Doska et al. (2012) found higher MUN values in winter than in summer in Brazil, because of the wide availability of grass rich in CP on Paraná plains that time of year.

The seasonal variation of MUN was confirmed in our study, as well. In contrast with previous research, mean MUN values in summer and autumn were the lowest of the year. However, the lowest MUN values were recorded in December and the highest in February. As shown in Table 4, MY and P in summer were, also, lower than in winter and spring. Additionally, MY and MUN were lower, while F and P were higher in autumn than in any other season.

Table 4. Mean milk yield, milk urea nitrogen (MUN) concentration, fat and protein percentage by season and month of the year

Season & Month	Environmental Temperature (°C)	Relative Humidity (%)	THI*	Milk yield (L)	SEM	MUN (mg/dL)	SEM	Protein (%)	SEM	Fat (%)	SEM
Spring	**15.1**	**66.5**	**59.0**	**27.8^{a}**	**.12**	**15.9^{a}**	**.04**	**3.47^{a}**	**.01**	**3.76^{a}**	**.01**
March	10.1	71.1	51.4	28.9	.16	16.2	.05	3.47	.01	3.76	.01
April	13.9	69.1	57.2	27.2	.25	15.5	.06	3.50	.01	3.84	.02
May	21.1	59.2	67.3	27.6	.24	15.6	.05	3.42	.01	3.67	.02
Summer	**26.9**	**46.8**	**73.9**	**26.8^{b}**	**.15**	**15.1^{b}**	**.04**	**3.36^{b}**	**.01**	**3.74^{a}**	**.01**
June	25.9	46.5	72.6	27.2	.22	15.5	.05	3.36	.01	3.74	.02
July	28.0	47.1	75.3	26.5	.20	14.7	.05	3.36	.01	3.73	.02
Autumn	**22.1**	**68.6**	**69.4**	**25.9^{c}**	**.14**	**14.3^{c}**	**.04**	**3.55^{c}**	**.01**	**3.99^{b}**	**.01**
September	21.9	62.4	68.6	25.9	.24	14.9	.06	3.48	.01	3.91	.03
October	17.8	64.1	62.8	25.5	.23	14.1	.07	3.56	.01	4.11	.02
November	11.3	79.2	53.0	26.4	.25	14.0	.06	3.60	.01	3.95	.03
Winter	**7.9**	**80.5**	**47.5**	**27.8^{a}**	**.11**	**15.8^{a}**	**.04**	**3.55^{c}**	**.01**	**3.88^{c}**	**.01**
December	9.7	85.0	50.2	27.6	.25	13.4	.06	3.61	.01	3.94	.02
January	6.6	82.3	45.3	27.6	.17	15.8	.05	3.56	.01	3.87	.02
February	7.3	74.2	47.0	28.1	.18	17.1	.07	3.50	.01	3.87	.02

Means with different superscripts within the same column differ (P ≤0.05).
**THI: Temperature-Humidity Index = (1.8*T+32) – [(0.55-0.0055*RH)*(1.8*T-26)], where T: temperature (in degrees Celsius) and RH: relative humidity (in %).*

All of the above investigated factors seem to influence the concentration of MUN. However, the observed differences were small, despite their statistical significance. In addition, the biological significance of these differences is not so important, as in each class of milk traits studied, mean MUN values were higher than target-values for dairy herds. Therefore, none of these non-nutritional parameters can be pointed out as the cause of the observed elevated MUN values in Greek dairy herds. This assumption is confirmed by the multiple regression model, in which test month, F, SCS, SNF, P, L, FY, MY and LY explained only 25.8% of MUN variation. Nutritional factors should be responsible for the remaining variation.

Dairy cows in Greek farms are kept indoors (either in free stall or in straw yards) the whole year and fed a total mixed ration, which usually contains forages in the form of silage and hay, and relatively high quantities of concentrates, with no access to pasture. Therefore, ration formulation and net energy/metabolizable protein balance are crucial factors. In periods of hot weather, dry matter intake (DMI) and MY decline. If CP of the diet is not adjusted upwards, crude protein intake (CPI) also declines and this can lead to lower MUN values. In periods of cold weather, DMI increases. If CP of the diet is not adjusted downwards, CPI also increases and this can lead to higher MUN values. However, due to the absence of more information on the feeding management and the calving distribution, we cannot provide definitive explanation for our observations.

The analysis of variance showed that cows with higher MY are expected to have higher MUN, but this was true only up to MUN values of 16 mg/dL. Rajala-Schultz and Saville (2003) did not found any significant relationship between MY and MUN in low-producing herds (<7,258 kg), while MY and MUN were positively correlated in high-producing herds (>10,433 kg). According to a model developed by Jonker et al. (1998), a 2,000 kg increase in MY per lactation would increase MUN by 2.85 mg/dL, but only up to 16.36 mg/dL. As already mentioned, reproductive performance is significantly impaired at MUN values >17 mg/dL (Ferguson et al., 1988; Melendez et al., 2000). Therefore, over-supplementation of diets with excessive CP and/or ruminally degradable CP will increase MUN and reproductive problems with no further benefit to MY.

CONCLUDING REMARKS

Mean MUN values in the Greek Holstein herds studied were elevated compared to herd target-values. Milk traits and test month significantly affected MUN concentration and should always be considered when evaluating MUN values; still, they could only explain about 1/4 of MUN variation. The remaining variation represents the effect of nutrition, usually CP and/or ruminally degradable CP content of the diet. Overfeeding CP results in increased MUN and could impair reproduction, with limited milk yield gain. Therefore, measuring MUN concentration is a valuable tool for the dietary management of modern dairy farms and should be routinely used as part of a health management protocol.

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REFERENCES

Aguilar M, Hanigan MD, Tucker HA, Jones BL, Garbade SK, McGilliard ML, Stallings CC, Knowlton KF, James RE (2012) Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. J Dairy Sci 95:7261-7268.

Arunvipas P, Dohoo IR, Vanleeuwen JA, Keefe GP (2003) The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. Prev Vet Med 59:83-93.

Bastin C, Laloux L, Gillon A, Miglior F, Soyeurt H, Hammami H, Bertozzi C, Gengler N (2009) Modeling milk urea of Walloon dairy cows in management perspectives. J Dairy Sci 92:3529-3540.

Baker LD, Ferguson JD (1993) Milk urea nitrogen as a metabolic indicator of protein feeding efficiency in dairy farms. In: Proceedings of 26th Annual Convention American association of Bovine Practitioners, Wageningen Academic Publishers, Albuquerque NM, pp 165-166.

Broderick GA, Clayton MK (1997) A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 80:2964-2971.

Butler WR (1998) Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 81:2533-2539.

Butler WR (2000) Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci 60/61:449-457.

Carlsson J, Bergstrom J, Pehrson B (1995) Variations with breed, age, season, yield, stage of lactation, and herd in the concentration of urea in bulk milk and individual cow's milk. Acta Vet Scand 36:245-254.

Clark JH, Spires HR, Davis CK (1978) Uptake and metabolism of nitrogenous components by the lactating mammary gland. Federation Proceedings 37:1233.

Doska MC, Ferreira da Silva DF, Horst JA, Valloto AA, Junior PR, de

Almeida R (2012) Sources of variation in milk urea nitrogen in Paraná dairy cows. Rev Bras Zootec 41(n.3):692-697.

Elrod CC, Butler WR (1993) Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J Anim Sci 71:694.

Fatehi F, Zali A, Honarvar M, Dehghan-banadaky M, Young AJ, Ghiasvand M, Eftekhari M (2012) Review of the relationship between milk urea nitrogen and days in milk, parity, and monthly temperature mean in Iranian Holstein cows. J Dairy Sci 95:5156-5163.

Ferguson JD, Blanchard T, Galligan DT, Hoshall DC, Chalupa W (1988) Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. J Am Vet Med Assoc 192(5):659-661.

Gaines WL (1928) An efficiency formula for dairy cows. Science, Volume 67, Issue 1735: 353-354.

Godden SM, Lissemore KD, Kelton DF, Leslie KE, Walton JS, Lumsden JH (2001a) Factors associated with milk urea nitrogen concentrations in Ontario dairy cows. J Dairy Sci 84:107-114.

Godden SM, Lissemore KD, Kelton DF, Leslie KE, Walton JS, Lumsden JH (2001b) Relationships between milk urea concentrations and nutritional management, production, and economic values in Ontario dairy herds. J Dairy Sci 84:1128-1139.

Gustafsson AH, Palmquist DL (1993) Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. J Dairy Sci 76:475-484.

Hof G, Vervoorn MD, Lenaers PJ, Tamminga S (1997) Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. J Dairy Sci 80:3333-3340.

Hojman D, Gips M, Ezra E (2005) Association between live body weight and milk urea concentration in holstein cows. J Dairy Sci 88:580-584.

Hojman D, Kroll O, Adin G, Gips M, Hanochi B, Ezra E (2004) Relationship between milk urea and production, nutrition and fertility traits in Israeli dairy herds. J Dairy Sci 87:1001-1011.

Hristov AN, Ropp JK (2003) Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. J Dairy Sci 86:2416-2427.

Johnson RG, Young AJ (2003) The association between milk urea nitrogen and DHI production variables in western commercial dairy herds. J Dairy Sci 86:3008-3015.

Jonker JS, Kohn RA, Erdman RA (1998) Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J Dairy Sci 81:2681-2692.

Jordan ER, Chapman TE, Holtan DW, Swanson LV (1983) Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing dairy cows. J Dairy Sci 66:1854.

Jordan ER, Swanson LV (1979) Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. J Anim Sci 48:1154.

Kamiya M, Kamiya Y, Tanaka M, Shioya S (2006) Effects of high ambient temperature and restricted feed intake on urinary and plasma 3-methylhistidine in lactating holstein cows. J Anim Sci 77:201-207.

Kohn RA, Kalscheur KF Russek-Cohen E (2002) Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. J Dairy Sci 85:227-233.

Leroy JL, Van Soom A, Opsomer G, Goovaerts IG, Bols PE (2008) Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high - yielding dairy cows. Reprod Domest Anim 43(5):623-32.

Licata E (1985) Subclinical mastitis and urea determination in cows' milk. Obiettivi e Doc-Vet 6:65-67.

Lucy M (2001) Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 84:1277-1293.

Melendez P, Donovan A, Hernandez J (2000) Milk urea nitrogen and infertility in Florida Holstein cows. J Dairy Sci 83:459-463.

Meyer PM, Machado PF, Coldebella A, Cassoli LD, Coelho KO, Mazza Rodrigues PH (2012) Validation of models for predicting milk urea nitrogen concentrations, estimating dry matter intake by the NRC (2001). Rev Bras Zootec 41(n.5):1271-1277.

Moore DA, Varga G (1996) BUN and MUN: urea nitrogen testing in dairy cattle. The Compendium on Continuing Education for the Practicing Veterinarian 18:712-720.

National Research Council (1971) A guide to environmental research on animals. National Academy of Science, Washington, DC.

Nousiainen J, Shingfield KJ, Huhtanen P (2004) Evaluation of milk urea nitrogen as a diagnostic of protein feeding. J Dairy Sci 87:386-398.

Oltner R, Emanuelson M, 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 12:47-57.

Rajala-Schultz PJ, Saville WJA (2003) Source of variation in milk urea nitrogen in Ohio dairy herds. J Dairy Sci 86:1653-1661.

Rhoads ML, Bilby TR, Rhoads RP, Baumgard LH (2008) Effects of nutrient metabolism and excess protein catabolism on dairy cow fertility. In: Proceedings of 24th Annual Southwest Nutrition and Management Conference. Arizona: Department of Animal Sciences-The University of Arizona.

Rzewuska K, Strabel T (2013) Effects of some non-genetic factors on concentration of urea in milk in Polish Holstein-Fresian cows. J Anim Feed Sci 22:197-203.

Schneider PL, Beede DK, Wilcox CJ (1988) Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. J Anim Sci 66:112-125.

Sonderman JP, Larson LL (1989) Effect of dietary protein and exogenous gonadotropin-releasing hormone on circulating progesterone concentrations and performance of Holstein cows. J Dairy Sci 72:2179.

Staples CR, Garcia-Bojalil CM, Oldick BS, Thatcher WW, Risco CA (1993) Protein intake and reproductive performance of dairy cows: a review, a suggested mechanism, and blood and milk urea measurements. In: Proceedings of 4th Annual Florida Ruminant Nutrition Symposium, pp 37-51, Univ. Florida, Gainesville.

Staples CR, Thatcher WW, Garcia-Bojalil CM, Lucy MC (1992) Nutritional influences on reproductive function. Van Horn; HH; Wilcox, CJ (eds). Large Dairy Herd Management, 382-392.

Staples CR, Thatcher WW (2001) Nutrient influences on reproduction of dairy cows. In: Proceedings of Mid-south Ruminant Nutrition Conference, pp 21-35.

Visek WJ (1984) Ammonia: its effects on biological systems, metabolic hormones, and reproduction. J Dairy Sci 67:481-498.

Wattiaux MA, Nordheim EV, Crump P (2005) Statistical evaluation of factors and interactions affecting Dairy Herd Improvement milk urea nitrogen in commercial Midwest dairy herds. J Dairy Sci 88:3020-3035.

Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH (2010) Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 93:644-655.