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Non-nutritional Factors Associated with Milk Urea Concentrations under Mediterranean Conditions

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Abstract: The objective of this study was to determine relationships between Milk Urea Nitrogen (MUN) concentrations and non-nutritional factors under Mediterranean conditions. Test-day records of 77 cows in a commercial dairy herd were used. Effects of test season, cow factors (parity, days in milk on test-day, calving season and calving year) and milk production traits on MUN concentrations were studied. Descriptive statistics were calculated and linear-regression models where cow effect was treated as random were used. Mean DIM, parity, MUN, milk yield, protein percentageand fat percentage were 245.97 d (SD=139.5), 3.71 (SD=1.8), 14.16 mg/dl (SD=7.9), 18.63kg/cow/d (SD=7.9), 3.64% (SD=0.9)and 3.33% (SD=0.5), respectively. There was a positive relationship between MUN and each of DIM on test-day ($P<0.0001$) and calving year ($P<0.0001$), whereas the relationship with parity was negative ($P<0.0021$). While there was a negative relationship between MUN and each of uncorrected milk yield ($P<0.028$), milk total protein concentration ($P<0.0001$)and total protein yield ($P<0.0001$), there was a positive relationship between MUN and fat yield ($P<0.0117$). MUN concentrations varied with calving season ($P<0.019$) but not with test-day season. However, there was no relationship of MUN concentrations with neither milk fat concentration nor milk yield after correcting for milk fat and total protein contents.

Key words: Dairy cow · milk urea nitrogen · non-nutritional factors

INTRODUCTION

Considerable interest has been developing for the use of milk urea nitrogen (MUN) concentrations as a means to assess dietary crude protein and energy supply relative to cows' requirements and to control pollution [1-6]. Such use has been promoted by recording MUN regularly while sampling milk. Using MUN to monitor the efficiency of dietary protein utilization requires the identification of factors other than nutritional ones that may influence milk urea [7]. MUN was found to vary with nutritional and non nutritional factors (season, parity, DIMand milk production). Some studies reported positive relationships [1, 7-9], whereas others have found either a negative [10], or no relationship [11] between MUN and milk yield. Results on associations between MUN and milk protein or fat percentages varied considerably among studies [9, 12]. A negative nonlinear association between MUN and milk fat and protein percentages and a significant negative nonlinear association with somatic cell score were found [12]. An inverse relationship between MUN grouped in categories and milk protein or fat percentage was reported by [9]. In Tunisia, individual cow milk samples are being routinely collected. But unlike other production data [13-15], MUN data have not yet been used in any investigation.

The objectives of this study were to determine MUN levels in a commercial herd under a Mediterranean climateand to evaluate associations between milk-test day MUN concentrations and season, cow factorsand milk traits.

MATERIALS AND METHODS

Data: The study was conducted on a commercial dairy herd in the north of Tunisia. There were 77 lactating Holstein cows with a yearly rolling milk production average of 6000 kg per cow. Data were obtained from test-day records collected by the National Centre for Genetic Improvement (OEP), Sidi Thabet, Tunis, between December 2004 and December 2006. During this period, cows were kept in free sheltered stalls. They had free access to water and were provided with two meals of roughage and up to 6 feedings/day of concentrate that

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was automatically distributed. They were milked twice a day at 04.00 h and 16.00 h. by an automatic machine. Milk samples were collected from the mixture of the morning and afternoon milkings. At the National Centre for Genetic Improvement, milk urea measurements were performed spectrophotometrically using an automated procedure with a Skalar Segmented Flow Analyzer. Milk was analyzed for fat and Crude Protein (CP) contents. Milk urea (mg/dl) was determined with an automated IR Fossomatic 4000 Milk Analyzer (Foss Netherlands).

Each cow observation included test-day dates, test-day milk yield, milk fat and protein percentages, DIM on test-day, calving dateand parity. Records with extreme values were excluded. A record was excluded if: DIM >600 days; milk protein <1.5% or >5%; milk fat was <1.5% or >6%; MUN tests were <0.1 mg/dl. Days in milk were grouped into 30-d classes, with those greater than 420 d grouped into one class. Milk yield was grouped by increments of 5 kg/d with those <= 10 kg/d grouped into one category and those >= 35kg/d in an upper category. Milk protein (MP) and milk fat (MF) percentages were each grouped into three categories: MP =3.0%, 3.0 <MP = 3.2% and MP> 3.2% for protein and MF =3.2%, 3.2<MF = 3.8% and MF>3.8% for fat. Test-day or calving seasons were: 1 = January to Mars, 2 = April to June, 3 = July to September, 4 = October to December.

Analysis

Variations of MUN concentrations with season and cow factors: Associations between test-day MUN concentrations and test season and cow factors (parity, DIM, calving season and calving year) were investigated using mixed linear regression [16]. Initially, variables were assessed individually (univariate mixed models) and the ones with P<0.25 were used in a single multivariate mixed model. A second mixed linear model was used to determine the association between MUN and DIM and parity categories. Test season, calving season and calving year were added to the model as covariates. Likewise relationships between MUN and either 1) DIM categories while controlling for DIM, parity, test season, calving season and calving year (covariates); 2) test-day season while controlling for DIM, parity, calving seasonand calving year; and 3) calving season while controlling for DIM, parity, test-day seasonand calving year were investigated. In all analyses, the cow effect was used as random and least squares means of factors or factor levels of interest were compared using the Tukey test.

Relationships between MUN concentrations and milk traits: Linear regression, using Proc Mixed in SAS [16], was used to investigate relationships between test-day MUN concentrations and each of the following measures of milk production: 1-uncorrected milk yield (kg/cow/d), 2-milk yield while controlling for fat and protein contents as additional covariates in the model (kg/cow/d), 3-weighed milk fat percentage, 4-weighed milk protein percentage, 5-weighed milk fat yield (kg/cow/d), 6-weighed milk protein yield (kg/cow/d). Test season and cow factors (parity, DIM on test-day, calving seasonand calving year) were included in all models as covariates. Relationships between MUN and milk yield categories and either protein or fat percentage categories and their interactions while controlling for DIM, parity, test season, calving seasonand calving year (covariates) were investigated. In all analyses, the cow effect was used as random and least squares means of factors or factor levels of interest were compared using the Tukey test.

RESULTS AND DISCUSSION

Analysed data: Of the 980 test records originally available, 64 records excluded from the analysis. Summary statistics describing herd and production parameters before and after edition are given in Table 1. Mean parity was 3.7 lactations (SD = 1.8; range = 1 to 8) which is higher than that reported in other studies: 2.46 (SE= 1.6) [17]. Average milk yield and MUN concentrations were 18.6 kg/cow (SD = 7.9; range = 2 to 43.3) and 30.4 mg/dl (SD = 17.0; range = 0.79 to 85.0), respectively. Milk yield was slightly lower than that reported nationwide for the same breed: 19.7 kg [14] but much lower than those found in reports on other Holstein populations [12, 17]. Overall, average cow-level MUN concentrations were similar to values reported in other studies [3, 12, 18]. Mean milk fat and protein percentages were 3.64 (SD=0.9) and 3.33% (SD=0.5), respectively. They were similar to values reported by [3, 19], but lower than those reported by [12].

Factors affecting variations of MUN concentrations: Results describing relationships between MUN concentrations and test season and cow factors (parity, DIM) are presented in Table 2. While there was a positive relationship between MUN and each of DIM on test-day (P<0.0001) and calving year (P<0.0001), the relationship was negative (P<0.0021) with parity. The season of calving was important (P<0.019) contrary to that of the test (P = 0.52) in explaining variations of MUN concentrations. [9] found conflicting parity effects for Holstein and Jersey cows. For Holsteins, the overall mean for the second parity was higher (P <0.0001) than that of first or third and greater parity means. Some studies found

Table 1: Test-day characteristics and production information for 77 cow-herd before (a) and after (b) edition

Item	N	Mean	SD	Minimum	Maximum
DIM	a980	264.10	167.70	1.0	966.0
	b916	245.97	139.49	11.0	600.0
Parity	a161	3.68	1.82	1.0	8.0
	b158	3.71	1.82	1.0	8.0
Milk yield (kg/cow/d)	a875	16.58	8.16	2.0	43.3
	b816	18.63	7.91	2.0	43.3
Milk fat (%)	a880	3.27	1.01	0.92	8.75
	b818	3.64	0.92	1.50	5.92
Total protein (%)	a842	2.87	0.45	1.98	5.29
	b783	3.33	0.45	1.98	4.70
Milk urea concentrations (mg/dl)	a980	30.61	17.12	0.79	97.00
Milk urea nitrogen (MUN) (mg/dl)	b916	30.39	17.00	0.79	85.00
	b916	14.16	7.92	0.37	39.61

Table 2: Multivariate regression model† describing the relationship between test-day MUN concentrations (mg/dl) and seasonand cow factors

Parameters	Estimate for mean MUN	SE	P value
DIM on test-day	0.0076	0.0014	<0.0001
Parity	-0.4577	0.1480	0.0021
Test-day season	-0.1156	0.1817	0.5247
Calving season	0.4627	0.1972	0.0192
Calving year	4.5696	0.2146	<0.0001

†Model controlled for random cow effect

Table 3: Least squares means of MUN concentrations by Days in Milk (DIM) categories and parity

DIM category	Lactation 1 Mean	Lactation 1 SE	Lactation 2 Mean	Lactation 2 SE	Lactation 3 Mean	Lactation 3 SE
≤30	15.93	4.55	8.25	4.56	7.97	1.60
31–60	9.96a	3.22	6.69ab	1.95	11.69ac	1.16
61-90	12.30	2.44	11.06	1.41	11.20	1.00
91-120	15.33	2.88	12.30	2.44	11.65	0.96
121-150	15.74a	2.04	7.17b	1.72	10.79b	0.94
151-180	15.40a	2.28	16.17ab	1.78	11.73ac	0.99
181-210	9.42	2.44	13.46	1.94	12.44	0.91
211-240	14.39	1.79	13.16	1.86	14.45	0.91
241-270	13.87a	2.15	16.22ab	1.86	12.07ac	0.94
271-300	17.94	2.15	15.29	1.78	15.61	1.02
301-330	19.75	2.43	18.21	2.14	17.30	1.09
331-360	18.23a	2.15	20.71ab	2.43	15.06ac	0.97
361-390	15.78	2.44	17.73	2.43	15.48	1.27
391-420	16.95	2.88	17.23	2.43	16.09	1.38
>420	19.79	1.16	18.09	1.33	18.37	0.87
Total/avg.	15.40a	0.71	14.12ab	0.62	13.46b	0.31

[a,b,c]Least squares means of DIM categories by parity with different superscripts differ at P = 0.05, [a,b,c] Least squares means of DIM categories among parities with different superscripts differ at P = 0.05

that MU was low in first lactation heifers [7, 12, 17, 19]. Others reported no significant association between parity and MUN [20]. It was also reported that multiparous cows had higher MUN concentrations than primiparous ones only when cows were in confinement housing rather than on pasture [8]. Likewise [3] suggested that first lactation cows would have higher MUN levels than mature ones.

Least squares means of MUN concentrations by DIM categories and parity are shown in Table 3. The overall mean decreased with parity. However, only first and third and greater parity means were different (P=0.013). Relationships between MUN concentrations and DIM categories are shown in Fig. 1. MUN concentrations were low (P <0.05) during the first 90 DIM compared to those for greater than 270 DIM categories. MUN concentrations between 90 and 270 DIM were intermediate. Results on variations of MUN with the stage of lactation are partially consistent with previous reports [8, 12]. [12] found that MUN concentrations were generally the lowest during the first 60 DIM, high between 60 and 150 DIMand again low following 150 DIM. [8] reported that MUN was the lowest immediately after calving, increased to a maximum between 3 and 6 months of lactationand slowly declined thereafter.

There was no significant association between MUN concentrations and test-day season (Table 2). However, the association was important (P<0.0001) between MUN and test-day season while controlling for DIM, parity, calving seasonand calving year in the model. Test-day seasons' least squares means are given in Table 4. Milk urea was the highest during the months of April to June and the lowest during the winter season (January to March). These results are consistent with those in [7, 17], but not with the reported increase of milk urea during the

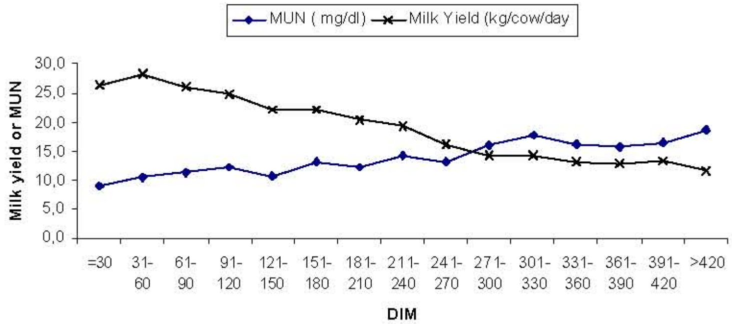

Fig. 1: Least squares means of milk yield and MUN concentrations by days in milk intervals

Table 4: Least squares means of MUN concentrations by test-day and calving seasons†

				Multiple comparison P value		
Item	Season	Mean	SE	A	B	C
Test-day	1	12.82	0.37			
	2	17.13	0.51	<0.0001		
	3	14.53	0.47	0.015	0.0007	
	4	13.67	0.42	0.375	<0.0001	0.499
Calving	1	10.95	0.49			
	2	13.58	0.64	0.005		
	3	15.24	0.37	<0.0001	0.115	
	4	16.19	0.52	<0.0001	0.009	0.398

A: Tukey's comparison of season 1 to others; B: Tukey's comparison of season 2 to 3 or 4; C: Tukey's comparison of season 3 to 4, †1 = January to March, 2 = April to June, 3 = July to September, 4 = October to December

Table 5: Regression models describing the relationship between test-day MUN concentration (mg/dl) and production variables

Model†	Variable	Estimate for mean MUN	SE	P value
1	Milk yield (kg/cow/d)‡	-0.0708	0.0321	0.028
2	Milk yield (kg/cow/d)§	0.0034	0.0359	0.9225
3	Milk fat (%)d¶	0.002044	0.004224	0.6286
4	Milk fat yield (kg/cow/d)¶	0.003243	0.001284	0.0117
5	Total protein (%)¶	-0.03012	0.002647	<0.0001
6	Total protein yield (kg/cow/d)d	-0.00586	0.001027	<0.0001

†Models control for random cow effect and for effects of season, parityand DIM, ‡Model without correction for milk fat and protein contents, §Model with correction for milk fat and total protein contents, ¶weighed by milk yield

summer season [8, 12, 21, 22]. The association between MUN concentrations and calving season was significant (P=0.019). Calving season least squares means adjusted for DIM, parity, test-day seasonand calving year are given in Table 4. Milk urea was the highest for fall and the lowest for winter calving. Comparison of milk yield and MUN concentrations curves (Fig. 1) and the negative association between milk yield and milk protein percentage suggest that there was an excess of N or insufficient energy supply as production declined. When energy supply is low, milk production is reduced causing less protein secretion in milk and consequently high urinary nitrogen and MUN [3, 5]. The highest MUN during the spring season may be caused by the use of more fresh forages.

Variation of MUN with milk traits: Relationships between MUN concentrations and milk traits are presented in Table 5. While there was a negative relationship between MUN and each of uncorrected milk yield (P<0.028), milk total protein concentration (P<0.0001)and total protein yield weighed by milk yield, there was a positive relationship between MUN and fat yield weighed by milk yield (P<0.0117). However, there was no relationship between MUN concentrations and neither milk fat content weighed by milk yield (P = 0.62) nor milk yield after correcting for milk fat and total protein contents (P = 0.92). MUN concentrations by milk yield and milk protein (MP) categories of MP =3.0%, 3.0<MP = 3.2%and MP>3.2% are given in Table 6. MUN concentrations were not different (P>0.05) among milk yield categories and decreased (P<0.05) from categories of low to high protein percentages. Positive relationships between MU and milk yield have been reported by [8, 9, 17, 19, 23]. There were also reports on significant nonlinear association [12], no relationship [11, 24], or a strong negative trend in the relationship between MUN and the uncorrected milk yield [1]. [1] suggested that MU concentrations remain moderate as long as levels of protein and energy were balanced relative to one another. The negative linear association between MUN and total protein percentage found in the current study is

Table 6: Least squares means and SE of MUN concentrations (mg/dl) by milk yield and protein percentage categories

	Protein percentage category								
	MP≤3.0%			3.0<PM≤3.2%			MP>3.2%		
Milk yield category†	N	Mean	SE	N	Mean	SE	N	Mean	SE
MY≤10	85	15.31	0.74	25	15.37	1.29	107	13.46	0.66
10<MY≤15	42	14.00	1.01	12	13.60	1.84	105	12.68	0.66
15<MY≤20	60	14.97	0.83	23	14.13	1.33	117	13.25	0.61
20<MY≤25	52	16.18	0.91	25	14.05	1 .28	86	12.94	0.70
25<MY≤30	50	15.17	0.94	22	14.88	1.37	36	13.15	1.09
30<MY≤35	24	19.28	1.33	11	14.36	1.96	11	10.59	1.95
MY>35	14	18.56	1.75	4	13.53	3.20	5	6.96	2.87
Total/avg	327	16.29[a]	0.45	122	14.27[b]	0.72	467	11.86c	0.56

consistent with those reported by [7, 9, 23] but not with those by [25, 26] who found no relationship between milk protein percentage and MUN, or [12] who found a negative nonlinear association between both parameters. In a related paper [1] a positive relationship (P<0.05) between herd mean MU and total protein percent was reported. Different feeding and management strategies, production levels, breed, or whether cow-level or herd-level analysis was conducted may all be sources of variation in reported results. The relationship between milk protein percentage and MUN differed between Holstein and Jersey cows at low milk protein percentages (<3.2%) [9], but were comparable at high milk protein percentages.

Changes of MUN concentrations with protein percentage suggest that protein percentage should be considered in addition to MUN concentrations as a monitoring tool to match dietary crude protein intake to cows' requirements, which optimizes the efficiency of dietary protein utilization, reduces nitrogen excretion into the environmentand improves fertility [6, 11, 18, 27]. These authors suggested that when herd-level mean milk protein was 3.0 to 3.2% and MUN concentration was 12 to 16 mg/dl, protein degradability fractions and net energy were most likely balanced. In this study (Table 6), cow-level mean MUN for each category of milk protein percentage and the average milk yield was within reported ranges. In agreement with [3], increased milk protein percentage category reduced MUN concentrations because more nitrogen intake was partitioned to milk protein.

Results on relationships between MUN concentrations and milk fat yield or milk fat percentage (Table 5) were not in agreement with previous reports [1, 3, 7, 9, 12, 28]. In a study using cow-level data [12], a negative nonlinear association between milk fat percentage and MUN was reported. When herd-level means were used [1, 7] found a positive relationship between both parameters. A change in milk fat of ±0.5% would change the estimated mean lactation MUN concentration by approximately ±1.70 mg/dl [3]. Increased milk fat percentage could have a negative effect on measured MUN [28]. In the current study, MUN concentrations were not associated with milk fat percentage while it was related to milk fat yield (Table 5).

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