

Milk Urea Nitrogen and Fertility in Dairy Farms

¹Mohammad Nourozi, ¹Alireza Heravi Moussavi, ²Mehran Abazari and
²Mohammad Raiisian Zadeh

¹Department of Animal Science, Ferdowsi University, Mashhad 91775-1163, Iran

²Khorasan Razavi Agricultural and Natural Resources Research Center,
Department of Animal Science, Agricultural Complex, Torogh, Mashhad 91735-488, Iran

Abstract: To evaluate the association between Milk Urea Nitrogen (MUN) and fertility of early lactation dairy cows, reproductive and productive data and MUN measurements from 10 dairy herds in northeast of Iran between 2005 and 2008 were analyzed by survival analysis and the Cox proportional hazards model. Days from calving to conception or to the end of the study were used as the outcome. The mean MUN values in the first 3 months of lactation were used to reflect the MUN status of a cow. Parity, mean milk yield, mean milk fat percentage and herd were included in the models as fixed effects. Cows with MUN values of 12-13.99 and 14-15.99 mg dL⁻¹ had higher fertility (15 and 8%, respectively) and cows with MUN value above 18 mg dL⁻¹ had lower fertility (10%) than cows with MUN value below 12 mg dL⁻¹ ($p < 0.0001$). The results indicate that increasing MUN levels above 18 mg dL⁻¹ appears to be negatively related to dairy cow fertility only in first parity ($p < 0.01$). It is also suggested that the levels of MUN that are adversely associated with fertility, might be < 12 and > 18 mg dL⁻¹.

Key words: Milk urea nitrogen, fertility, dairy cow, dairy herds, energy, Iran

INTRODUCTION

One of the important factors in low conception rate of dairy cows in Iran is problems due to undesirable dietary protein intake or imbalance between Fermentable Metabolism Energy (FME) and Effective Rumen Degradable Protein (ERDP).

Nutrition management may be an important means to improve dairy cow reproductive performance (Ferguson and Chalupa, 1989). It has been shown that Blood Urea Nitrogen concentration (BUN) is a sensitive indicator of the balance between amount and availability of digestible crude protein and energy fed to ruminants (Kenny *et al.*, 2001, 2002) and BUN helps measure efficiency of protein utilization (Manston *et al.*, 1975; Broderick and Clayton, 1997; Jonker *et al.*, 2002; Kohn *et al.*, 2002).

Kinetic analysis has suggested that Milk Urea Nitrogen concentration (MUN) is a reasonable indicator of BUN (Baker and Ferguson, 1993). Because milk samples are routinely collected from many dairy farms by Dairy Herd Improvement (DHI) organizations as part of production monitoring schemes, MUN testing is also more practical, rapid and inexpensive than BUN for routine nutritional monitoring (Baker and Ferguson, 1995). Milk Urea Nitrogen (MUN) is a by-product of dairy cattle protein metabolism and a reflection of urinary nitrogen

excretion (Jonker *et al.*, 1998). Several studies reported the negative effects of blood or milk urea nitrogen on reproductive performance in dairy cows and suggested that overfeeding CP caused reproductive stress (Ferguson *et al.*, 1993; Rajala-Schultz and Saville, 2001), although others have reported reduced conception risks only when MUN was either very low (< 7 mg dL⁻¹) or very high (> 17.6 mg dL⁻¹) (Miettinen, 1991; Pehrson *et al.*, 1992; Carlsson and Pehrson, 1993). But some other studies, revealed no significant or unequivocal relationship between urea-nitrogen concentrations and reproduction (Barton *et al.*, 1996; Kenny *et al.*, 2001, 2002; Godden *et al.*, 2001).

Butler *et al.* (1996) showed that conception rates of lactating dairy cows decreased approximately 20 percentage points when Plasma Urea Nitrogen (PUN) or MUN concentrations exceeded 19 mg dL⁻¹. Guo *et al.* (2004) reported that MUN had minimal effect on conception rate but was associated with greater days open among high-producing herds. Research demonstrating the association between excess dietary protein intake, elevated PUN/MUN and poor reproductive performance has led to the routine use of MUN analysis for monitoring protein nutrition in dairy herds (Hammon *et al.*, 2005). Disagreement among the above studies might be due in part to differences in study

design. Reproductive variables involving multiple breedings would be less likely to have a detectable effect from MUN because the many other causes of reproductive problems might be overwhelming any signal associated with MUN.

Therefore, the objective was to investigate the relationship between milk urea nitrogen and days open in early lactating cows in some commercial Holstein dairy herds in northeast of Iran. Because detoxification of ammonia to urea requires energy and therefore the negative-energy balance that affects many dairy cows in early lactation could be exacerbated by the costs of this detoxification making a relationship between MUN and days open more likely to be present than with the success of other breedings.

MATERIALS AND METHODS

The data for this study came from the 10 dairy herds that were randomly selected from dairy cow farms in the countryside of Mashhad. Milk yield and reproductive records from March 2005 to March 2008 were considered. Milk samples were collected monthly during normal milking time, 3 times a day. Information on the interval from service to conception, date of calving, milk yield and milk fat percentage was extracted from records provided by Khorasan Razavi Deputy of Livestock Affairs.

Mean MUN values over the first 3 months postpartum for each cow were measured and based on mean MUN value, cows were grouped into 5 classes including: <12, 12-13.99, 14-15.99, 16-17.99 and >18 mg dL⁻¹. According to the parity number, cows were in classes 1, 2, 3 and 4 or more parity and according to the milk yield over the first 12 week postpartum were grouped in <20, 20-29.99, 30-39.9 and ≥40 kg day⁻¹. Cows were also grouped based on milk fat percentage by six categories of <2.8, 2.8-2.99, 3-3.19, 3.2-3.39, 3.4-3.59 and ≥3.6%. Days from parturition to successful AI were considered as fertility potential for each cow in each lactation. During 3 years, 5207 parturitions from 10 dairy cow farms were monitored in this study.

MUN analysis: The MUN content was also determined by enzymatic assay. Milk was centrifuged at 1200 x g and the fat was removed. The defatted milk was then thoroughly mixed and deproteinized by mixing 0.2 mL of defatted milk with 1.8 mL of cold TCA and allowing the mixture to stand for 5 min. The sample was then centrifuged at 1200 x g for 5 min at 20°C and 0.2 mL of the clear supernatant was analyzed for MUN using a colorimetric diacetyl monoxine procedure (Sigma Chemical Co., St. Louis, MO). To improve accuracy and precision of analysis in each series of analysis, four milk samples were prepared as control

and urea (2.5 mg dL⁻¹) was added to two of them. Difference between mean MUN values of these controls was used to correct the data.

Statistical analysis: In order to evaluate the relationship between MUN and fertility in dairy cows, survival analysis with the Cox proportional hazard model of SAS Institute Inc. (1994) was used. In this model, outcome variable was days from calving to conception or to the end of the study or to the last test day from which data were available for a cow. A cow could have been censored if she had been bred but had not conceived or had suffered embryonic loss prior to confirmation of the pregnancy.

Parity, mean milk yield and mean milk fat percent were included in the model as fixed effects. Herd effect as a constant effect was also included in all models to reflect management and food program differences. Survival analysis allows the inclusion of data from cows that did not experience the event of interest during the study period (did not conceive or had not been reported pregnant) and thus the loss of information due to incomplete records can be minimized.

The Cox model is semi parametric; the effects of the covariates on the event times (in this study, time to conception or to the end of the study) are of parametric form but the underlying survivor function (distribution of event times) need not to be specified.

The proportional hazards model describes a hazard of some events (in this study, conception) for an individual *i* at any time *t* and it can be written as a product of two factors: a baseline hazard function $\lambda_0(t)$ that is left unspecified (except that it cannot be negative) and a linear function of a set of *k* fixed covariates (x_1-x_k), which is then exponentiated (Allison, 1995):

$$H_i(t) = \lambda_0(t) \exp(\beta_1 x_{i1} + \dots + \beta_k x_{ik})$$

$H_i(t)$ = Hazard of conception for an individual (*i*) at any time (*t*)
 $\lambda_0(t)$ = Baseline hazard function
 $\beta_1 x_{i1} + \dots + \beta_k x_{ik}$ = Linear function of a set of *k* fixed covariates $\tilde{x}_i x_k$

The earlier survival analysis model analyzed 4100 calvings from over 5207 and censored 1107 ones either due to pregnancy loss or due to the lack of enough information on pregnancy status.

RESULTS AND DISCUSSION

Main characteristics of the herds surveyed in this study are shown in Table 1. Mean MUN value in the first 3 months postpartum was 17.7 mg dL⁻¹. Hazard ratio for

Table 1: Main characteristics of herds surveyed

Characteristics	Values
No. of herds	10.00
No. of parturition records ¹	5207.00
Mean milk yield in the first 3 months postpartum (kg day ⁻¹)	33.87
Mean percent of milk fat in the first 3 months postpartum (%)	3.14
Mean MUN content in the first 3 months postpartum (mg dL ⁻¹)	17.70

¹ Some cows were recorded until parity 3 and 4

Table 2: Hazard Ratio¹ (HR) and 95% confidence interval² of the effect of covariates on days open in 4100 parturitions

Covariate	HR	95% confidence intervals		Probability
		Low	High	
Lactation				
1	1	-	-	
2	0.989	0.934	1.047	
3	1.010	0.956	1.068	
≥4	1.035	0.973	1.099	0.5200
Mean milk yield (kg day⁻¹) (First 3 months of lactation)				
<20	1	-	-	
20-29.99	1.236**	1.100	1.389	
30-39.99	0.949	0.889	1.012	
≥40	0.942*	0.891	0.996	0.0068
Fat percent (First 3 months of lactation)				
<2.80	1	-	-	
2.80-2.99	1.135**	1.072	1.200	
3.00-3.19	0.991	0.916	1.073	
3.20-3.39	0.968	0.894	1.049	
4.40-3-59	0.961	0.882	1.046	
≥3.6%	0.990	0.896	1.094	0.0011
Mun (mg dL⁻¹) (First 3 months of lactation)				
<12	1	-	-	
12-13.99	1.150**	1.074	1.231	
14-15.99	1.082**	1.005	1.165	
16-17.99	1.038	0.969	1.112	
≥18	0.906*	0.844	0.973	0.000001

¹HR>1: Positive effect, HR = 1: no effect and HR<1: negative effect.
²Number 1 in the 95% confidence intervals shows the absence of significant relationship **Significant positive effect. * Significant negative effect

different groups of covariates relative to their level 1 with 95% confidence intervals is shown in Table 2. Effect of herd entered the model but hazard ratio for it is not shown in the Table 2.

Generally, lactation number had no effect on days open whereas it was affected significantly by mean milk yield, milk fat percent and MUN content. Positive effects of the mean milk yield on fertility were obtained (p<0.01) for means between 20 and 29.99 kg day⁻¹ and negative effects were found (p<0.01) for means ≥40 kg day⁻¹. In the case of milk fat, only group 2 among 6 groups had significant positive effect on fertility as a whole relative to the group 1. Relative to the level 1, 2 and 3 of MUN had significant positive effect and its level 5 had significant negative effect on fertility.

Relationship between lactation number and fertility:

Hazard ratios concerning the effect of different lactations on fertility, didn't show any significant relationship between different lactations and fertility. This result may reveal the equal prevalence of reproductive problems between lactations. Of course from the point of view of factors causing decreased fertility, there are probably significant differences between factors that affect fertility in different lactations.

Relationship between milk yield level and fertility:

As shown in Table 2, cows that produced 20-29.99 kg day⁻¹ milk in the first three months postpartum had 24% higher fertility than cows with milk yield below 20 kg day⁻¹ (p<0.01). Of course for cows with milk yield >40 kg day⁻¹, fertility rate was 6% less than cows of first group (Milk yield: <20 kg day⁻¹). The requirements for maintenance, growth and milk production in cows with milk yield between 20 and 20.99 kg day⁻¹ may be completely supplied by common rations utilized in the farms and it seems that this group had not been under any nutritional stress. Thus, this group had greater (p<0.01) fertility than the first group. Lower feed intake may account for the lower milk production of the first group. Fertility problems in cows producing >40 kg day⁻¹ milk were more than other groups because majority of them were at peak lactation and maximum negative energy balance. Butler (2000) suggested that negative energy balance during the first 3-4 weeks postpartum is highly correlated with the days to first ovulation. High milk yield in cows is dependent on high levels of dietary protein as well as energy. Depending upon protein quantity and composition, serum concentrations of progesterone may be lowered, the uterine environment altered and fertility decreased (Butler, 1998).

Relationship between milk fat percent and fertility:

Fertility rate in cows with milk fat percent between 2.8 and 2.99 was 14% higher than that of cows with milk fat percent under 2.8%. No significant relationship between milk fat percent and fertility was observed in other levels of milk fat percent. Of course milk fat percent below 2.8% could be attributed to over consumption of concentrate and also impaired rumen fermentation which have detrimental to reproductive functions and overall cow health. Cows with digestive disorders are more likely to have lower reproductive efficiency compared with healthy cows.

Relationship between MUN and fertility:

Cows with MUN concentrations between 12 and 13.99 mg dL⁻¹ and also cows with MUN levels between 14 and 15.99 mg dL⁻¹ had

15 and 8%, respectively higher ($p < 0.0001$) fertility than cows with MUN levels $< 12 \text{ mg dL}^{-1}$. A reduction ($p < 0.001$) of about 10% in fertility was observed in cows with MUN levels over 18 mg dL^{-1} . This suggests that without consideration to other factors in order to reach maximum fertility, rations should be balanced in a manner that MUN levels range between 12 and 16 mg dL^{-1} . In a similar study, Rajala-Schultz and Saville (2001) found that cows with MUN levels below 10.0 were 2.4 times more likely and cows with MUN levels between 10.0 and 12.7 mg dL^{-1} were 1.4 times more likely to be confirmed pregnant than cows with MUN values above 15.4 mg dL^{-1} .

In the study of Gustafsson and Carlsson (1993), MUN concentrations between 10 and 16 mg dL^{-1} were associated with the fewest days to first service (~80 days) with days to first service increasing to 128 days when MUN averaged 20 mg dL^{-1} . Several hypotheses have been proposed to explain why overfeeding protein might negatively influence reproductive performance. One that has received some attention is that the uterine environment may be adversely modified by overfeeding protein so that the normal processes leading to fertilization, embryo development and implantation of the conceptus are hampered. Associated with the elevated concentrations of uterine urea is a decreasing uterine pH (Elrod *et al.*, 1993). In addition to pH changes, ion concentrations (K, Mg and P) were reported to decrease when CP of diets increased from 12-23% (Jordan *et al.*, 1983). Because the uterine environment influences embryo development, these changes may compromise normal fertility processes.

Another theory to explain the negative reproductive performance states that the energy costs of detoxifying large amounts of ammonia to urea may aggravate an existing energy shortage postpartum such that metabolic attention is diverted away from ovarian activity (Staples *et al.*, 1990). Energy expended for ammonia detoxification has been reported to be $7.2 \text{ kcal of ME g}^{-1}$ of excess N (NRC, 1989).

That high dietary protein may mediate its negative effects on reproduction through energetic mechanisms is supported by the research of Garcia-Bojalil *et al.* (1998). Liver function may be compromised by excess ammonia such that important energy transactions are inhibited (Overton *et al.*, 1999).

HR values for the effect of different levels of MUN on fertility across lactations are shown in Table 3. MUN values above 18 mg dL^{-1} had negative effect ($p < 0.01$) only on first parity cows relative to MUN level 1.

Table 3: Hazard Ratio¹ (HR) and 95% confidence interval² for the effect of different levels of MUN on fertility of cows across lactations

Lactation	MUN level ³	HR	95% confidence intervals		Probability
			Low	High	
1	1	1	-	-	0.0026
	2	1.114	0.990	1.254	
	3	1.134	0.993	1.295	
	4	1.048	0.924	1.189	
	5	0.865*	0.760	0.984	
2	1	1	-	-	0.1329
	2	1.069	0.917	1.239	
	3	1.115	0.942	1.308	
	4	1.000	0.863	1.154	
	5	0.950	0.814	1.102	
3	1	1	-	-	0.0456
	2	1.221	1.036	1.430	
	3	0.920	0.764	1.097	
	4	1.095	0.929	1.282	
	5	0.895	0.747	1.063	
≥4	1	1	-	-	0.0618
	2	1.107	0.979	1.215	
	3	1.072	0.941	1.221	
	4	0.998	0.880	1.131	
	5	0.950	0.837	1.077	

¹HR>1: positive effect; HR = 1: no effect; HR<1: negative effect. ²Number 1 in the 95% confidence intervals shows the absence of significant relationship; ³1: $< 12 \text{ mg dL}^{-1}$; 2: $12\text{-}13.99 \text{ mg dL}^{-1}$; 3: $14\text{-}15.99 \text{ mg dL}^{-1}$; 4: $16\text{-}17.99 \text{ mg dL}^{-1}$; 5: $\geq 18 \text{ mg dL}^{-1}$. * Significant negative effect

Relationship between MUN and fertility in different parities: With regard to the effect of increased MUN on fertility as one of factors associated with reduced conception rate, researchers observed that negative effect of MUN on fertility was significant only in first lactation cows. In this group if ration was balanced in a manner that increased MUN level to $> 18 \text{ mg dL}^{-1}$, then fertility rate would be reduced ($p < 0.01$) by 14%. Generally, first parity cows are smaller in size and consume less feed than mature ones, besides, both stress of parturition and negative energy balance associated with it, cause difference between the nutritional requirements of this group with that of others.

Thus, the intensified negative energy balance and also excess of ammonia in rumen that subsequently result in increased need of energy to deaminate ammonia to urea in liver cause increased incidence of reproduction problems compared to other lactation groups in the case of increasing MUN concentration to over of 18 mg dL^{-1} . This matter reveals the importance of feeding the first lactation cows separate from others. In a study by Jonker *et al.* (1998), first lactation cows had higher MUN concentrations than mature cows. Besides, Elrod and Butler (1993) reported an increase in embryo losses for heifers fed a diet high in degradable protein at restricted energy intake. Therefore, the combined effects of excess RDP and energy status might explain why the embryo quality of lactating cows was compromised. Johnson and Young (2003) reported that for Holsteins, the overall mean for the second-parity group was higher than first or third and greater parity, likewise in a study by Godden *et al.* (2001), MUN was lower for first parity cows than that of

Table 4: Hazard Ratio¹ (HR) and 95% confidence interval² for the effect of different levels of MUN on fertility of cows across production groups

Milk yield level	MUN level ³	HR	95% confidence intervals		Probability
			Low	High	
<20 kg day ⁻¹	1	1	-	-	0.1718
	2	1.120	0.835	1.481	
	3	0.721	0.490	1.020	
	4	1.218	0.856	1.683	
	5	0.843	0.546	1.232	
20-29.99 kg day ⁻¹	1	1	-	-	0.1286
	2	1.141	1.007	1.293	
	3	0.977	0.848	1.120	
	4	1.000	0.881	1.136	
	5	0.997	0.870	1.137	
30-39.99 kg day ⁻¹	1	1	-	-	0.00001
	2	1.092	0.990	1.205	
	3	1.202**	1.080	1.337	
	4	1.027	0.931	1.134	
	5	0.871*	0.785	0.966	
≥40 kg day ⁻¹	1	1	-	-	0.0770
	2	0.145	0.976	1.336	
	3	1.017	0.858	1.197	
	4	1.073	0.910	1.256	
	5	0.898	0.769	1.042	

¹HR>1: no effect; HR = 1: positive effect; HR<1: negative effect. ²Number 1 in the 95% confidence intervals shows the absence of significant relationship; ³1: <12 mg dL⁻¹; 2: 12-13.99 mg dL⁻¹; 3: 14-15.99 mg dL⁻¹; 4: 16-17.99 mg dL⁻¹; 5: ≥18 mg dL⁻¹. ** Significant positive effect. *Significant negative effect

mature ones. From these studies and the study, it can be concluded that results are different depending on the kinds of feedstuffs, feeding rationing and feeding system in each region under study. Probably in the farms of this study, imbalance of energy and protein and also negative effect of feeding excessive protein on fertility have been more prominent in first parity cows than other cows. The data for the effect of different levels of MUN on fertility of cows across production groups are shown in Table 4. HR values for different levels of milk yield and MUN shows that the effects of MUN on fertility were significant only in cows with production of 30-39.99 kg day⁻¹ milk so that MUN ≥ 18 mg dL⁻¹ compared to MUN ≤ 12 mg dL⁻¹ significantly reduced fertility and the highest (p<0.0001) fertility was observed in cows with MUN between 14-15.99 mg dL⁻¹.

Relationship between MUN and fertility in different levels of milk production: MUN level above 18 mg dL⁻¹ caused significant decrease (13%) in fertility rate only in cows with production of 30-39.99 kg day⁻¹ milk and in this range of milk production better fertility rate was obtained when MUN concentration was 14-15.99 mg dL⁻¹.

Godden *et al.* (2001) also reported a positive nonlinear association between cow-level MUN and milk yield. The positive association between MUN and milk yield may be attributed to increased milk production which resulted from increased levels of dietary protein fed. Supplemental protein may increase milk yield by

Table 5: Hazard Ratio¹ (HR) and 95% confidence interval² for the effect of different levels of MUN on fertility of cows grouped by levels of milk fat

Level of milk fat (%)	MUN level ³	HR	95% confidence intervals		Probability
			Low	High	
<2.80	1	1	-	-	0.0013
	2	1.153	1.025	1.296	
	3	1.077	0.945	1.228	
	4	1.053	0.939	1.180	
	5	0.866*	0.767	0.979	
2.80-2.99	1	1	-	-	0.5479
	2	1.110	0.900	1.355	
	3	1.092	0.886	1.332	
	4	0.915	0.740	1.119	
	5	0.977	0.792	1.191	
3.00-3.19	1	1	-	-	0.2468
	2	1.237	1.013	1.498	
	3	1.029	0.816	1.278	
	4	0.924	0.746	1.131	
	5	0.875	0.713	1.064	
3.20-3.39	1	1	-	-	0.9440
	2	0.954	0.752	1.191	
	3	0.997	0.777	1.257	
	4	1.091	0.885	1.332	
	5	0.977	0.779	1.208	
3.40-3.59	1	1	-	-	0.8337
	2	0.983	0.753	1.259	
	3	1.018	0.786	1.297	
	4	1.100	0.841	1.412	
	5	1.009	0.800	1.260	
≥3.60	1	1	-	-	0.0001
	2	1.152	1.015	1.307	
	3	1.122	0.968	1.295	
	4	1.083	0.937	1.246	
	5	0.914	0.780	1.064	

¹HR>1: positive effect; HR=1: no effect; HR<1: negative effect. ²Number 1 in the 95% confidence intervals shows the absence of significant relationship. ³1: <12 mg dL⁻¹; 2: 12-13.99 mg dL⁻¹; 3: 14-15.99 mg dL⁻¹; 4: 16-17.99 mg dL⁻¹; 5: = 18 mg dL⁻¹. *Significant negative effect

providing more amino acid for milk protein synthesis, by increasing the available energy through deamination of amino acid or by altering the efficiency of utilization of absorbed nutrients. In this study, harmful effect of MUN on fertility was only observed in first parity cows. Thus, we may conclude that first lactation cows with high production (generally about 30-40 kg day⁻¹) have been grouped with high producing cows of the herd that receive high protein concentrate so MUN levels were increased and this excess MUN caused more reduction in pregnancy rate of first lactation cows compared with older ones.

Grouping the cows according to milk fat percentage and effects of different levels of MUN on fertility in these groups are shown in Table 5. The greatest negative effect of high levels of MUN (≥18 mg dL⁻¹) was observed in cows with milk fat percentage below 2.8%.

Relationship between MUN and fertility in different levels of milk fat: Fertility of cows with milk fat below 2.8% was more sensitive to the harmful effects of MUN compared

with other groups and when MUN level was $>18 \text{ mg dL}^{-1}$, their pregnancy rate was decreased ($p < 0.01$) compared with the first group. This may be related to overfeeding of concentrate or imbalance of forage and concentrate that leads to the reduced milk fat as a result of rumen pH reduction. Johnson and Young (2003) reported that within the normal physiological range of milk fat percentages for each breed, MUN concentration change was very small but as milk fat percentage increased MUN concentration decreased. Godden *et al.* (2001) reported a negative nonlinear association between MUN and milk fat. Broderick and Clayton (1997) also reported a negative relationship between MUN and milk fat.

Jonker *et al.* (1998) predicted that a change in milk fat of ± 0.5 percentage units would change the estimated mean lactation MUN concentration by approximately $\pm 1.70 \text{ mg dL}^{-1}$. The relationship between milk fat and MUN may be an indirect result of nutritional variables or a direct negative effect of milk fat on MUN (Carlsson and Bergstrom, 1994).

CONCLUSION

The results of this study show that the relationship between milk urea nitrogen and fertility is more significant for first parity cows and also for cows with the production of $30\text{-}40 \text{ kg day}^{-1}$ milk. In the farms of this study, first parity cows which are high yielding are usually grouped with mature high yielding cows and since in competition with mature cows, in early lactation they can't consume feed proportional to their needs have more weight loss and because of higher mobilization of protein and converting the amino acids to urea in the liver they have higher MUN than that of older cows, therefore negative effect of excessive MUN on fertility was more prominent in these cows.

Moreover, it could be that the first parity cows are not able to adapt with excessive urea in their body as well as older cows and this high intake of protein led to increase of urea concentration to $>18 \text{ mg dL}^{-1}$ has caused a significant reduction in fertility rate of this group compared with other groups.

This does not imply that fertility problems are more in first parity cows but it shows the higher sensitivity to the increased body urea in these cows. Overall, for maximizing the fertility rate in first parity cows, it is necessary to maintain MUN in the level of $12\text{-}16 \text{ mg dL}^{-1}$ by supplying more energy sources for rumen microbes and/or by decreasing protein intake.

ACKNOWLEDGEMENTS

The researchers thank the Khorasan Razavi Agricultural and Natural Resources Research Center for financial support.

REFERENCES

- Allison, P.D., 1995. Survival Analysis Using the SAS System: A Practical Guide. SAS Institute Inc., Cary NC, ISBN-13: 9781555442798, pp: 292.
- Baker, L.D. and J.D. Ferguson, 1993. Milk urea nitrogen as a metabolic indicator of protein feeding efficiency on dairy farms. Proceedings of 26th Annual Convention American Association of Bovine Practitioners, (ACAABP'93), Wageningen Academic Publishers, Albuquerque NM, pp: 165-166.
- Baker, L.D. and J.D. Ferguson, 1995. Response in urea and true protein of milk to different protein feeding schemes for dairy cows. *J. Dairy Sci.*, 78: 2424-2434.
- Barton, B.A., H.A. Rosario, G.W. Andersson, B.P. Grindle and D.J. Carroll, 1996. Effects of dietary crude protein, breed, parity and health status on the fertility of dairy cows. *J. Dairy Sci.*, 79: 2225-2236.
- Broderick, G.A. and M.K. Clayton, 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.*, 80: 2964-2971.
- Butler, W.R., 1998. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J. Dairy Sci.*, 81: 2533-2539.
- Butler, W.R., 2000. Nutritional interactions with reproductive performance in dairy cattle. *Anim. Reprod. Sci.*, 60-61: 449-457.
- Butler, W.R., J.J. Calaman and S.W. Beam, 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J. Anim. Sci.*, 74: 858-865.
- Carlsson, J. and B. Pehrson, 1993. The relationships between seasonal variations in the concentration of urea in bulk milk and the production and fertility of dairy herds. *Zentralbl. Veterinarmed. A*, 40: 205-212.
- Carlsson, J. and J. Bergstrom. 1994. The diurnal variation of urea in cow's milk and how milk fat content, storage and preservation affects analysis by a flow injection technique. *Acta. Vet. Scand.*, 34: 67-77.
- Elrod, C.C. and W.R. Butler, 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *J. Anim. Sci.*, 71: 694-701.
- Elrod, C.C., M. van Amburgh and W.R. Butler, 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *J. Anim. Sci.*, 71: 702-706.
- Ferguson, J.D. and W. Chalupa, 1989. Impact of protein nutrition on reproduction in dairy cows. *J. Dairy Sci.*, 72: 746-766.
- Ferguson, J.D., D.T. Galligan, T. Blanchard and M. Reeves, 1993. Serum urea nitrogen and conception rate: the usefulness of test information. *J. Dairy Sci.*, 76: 3742-3746.

- Garcia-Bojalil, C.M., C.R. Staples, C.A. Risco, J.D. Savio and W.W. Thatcher, 1998. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: Reproductive responses. *J. Dairy Sci.*, 81: 1385-1395.
- Godden, S.M., D.F. Kelton, K.D. Lissemore, J.S. Walto and K.E. Leslie *et al.*, 2001. Milk urea testing as a tool to monitor reproductive performance in Ontario dairy herds. *J. Dairy Sci.*, 84: 1397-1406.
- Guo, K., E. Russek-Cohen, M.A. Varner and R.A. Kohn, 2004. Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *J. Dairy Sci.*, 87: 1878-1885.
- Gustafsson, A.H. and J. Carlsson, 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.
- Hammon, D.S., G.R. Holyoak and T.R. Dhiman, 2005. Association between blood plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactation dairy cows. *Anim. Reprod. Sci.*, 86: 195-204.
- Johnson, R.G. and A.J. Young, 2003. The association between milk urea nitrogen and DHI production variables in western commercial dairy herds. *J. Dairy Sci.*, 86: 3008-3015.
- Jonker, J.S., R.A. Kohn and J. High, 2002. Use of milk urea-nitrogen to improve dairy cow diet. *J. Dairy Sci.*, 85: 939-946.
- Jonker, J.S., R.A. Kohn and R.A. Erdman, 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.*, 81: 2681-2692.
- Jordan, E.R., T.E. Chapman, D.W. Holtan and L.V. Swanson, 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high producing post partum dairy cows. *J. Dairy Sci.*, 66: 1854-1862.
- Kenny, D.A., M.P. Boland, M.G. Diskin and J.M. Sreenan, 2001. Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentration and embryo survival in heifers. *Anim. Sci. Abstr.*, 73: 501-512.
- Kenny, D.A., M.P. Boland, M.G. Diskin and J.M. Sreenan, 2002. Effect of rumen degradable protein with or without fermentable carbohydrate supplementation on blood metabolites and embryo survival in cattle. *Anim. Sci.*, 74: 529-537.
- Kohn, R.A., K.F. Kalscheur and E. Russek-Cohen, 2002. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. *J. Dairy Sci.*, 85: 227-233.
- Manston, R., A.M. Russell, J.M. Payne and S.M. Dew, 1975. The influence of dietary protein upon blood composition in dairy cows. *Vet. Rec.*, 96: 497-502.
- Miettinen, P.V.A., 1991. Correlation between energy balance and fertility in Finnish dairy cows. *Acta Veterinaria Scandinavica*, 32: 189-196.
- NRC, 1989. *Nutrient Requirements of Dairy Cattle*. 6th Rev. Edn., National Academy of Sciences, Washington DC, ISBN: 0309059933, pp: 90-110.
- Overton, T.R., J.K. Drackley, C.J. Ottemann-Abbamonte, A.D. Beaulieu, L.S. Emmert and J.H. Clark, 1999. Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. *J. Anim. Sci.*, 77: 1940-1951.
- Pehrson, B., K.P. Forshell and J. Carlsson, 1992. The effect of additional feeding on the fertility of high-yielding dairy cows. *Zentralbl. Veterinarmed. A*, 39: 187-192.
- Rajala-Schultz, P.J. and W.J.A. Saville, 2001. Association between milk urea nitrogen and fertility in Ohio dairy cows. *J. Dairy Sci.*, 84: 482-489.
- SAS Institute Inc., 1994. *SAS/STAT Software: Changes and Enhancements*. SAS Institute Inc., Cary NC, ISBN: 1-55544-643-4.
- Staples, C.R., W.W. Thatcher and J.H. Clark, 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *J. Dairy Sci.*, 73: 938-947.