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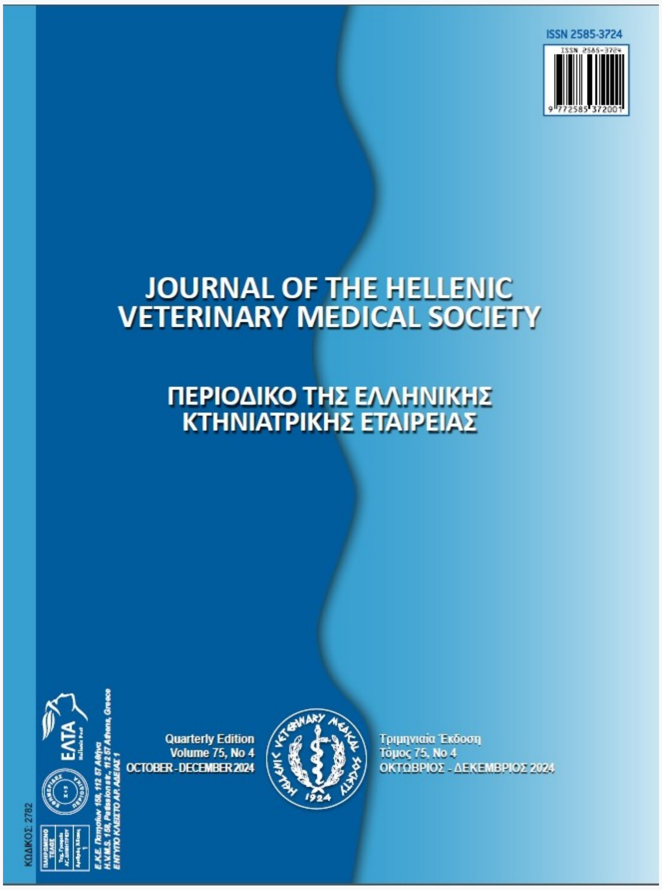

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Research article
Ερευνητικό άρθρο

A study investigating the relationship between milk urea nitrogen and the reproductive performance of Holstein cows

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ABSTRACT: Since protein is a fundamental component of dairy cows' diet, as well as its metabolites, it is critical to pay attention to milk urea nitrogen (MUN) in order to control the protein concentration in diet because it is one of the most prevalent compounds in milk. Hence, the present research evaluated MUN and its relationship with some reproductive performances and fertility of Holstein dairy cows. There were four groups of data concerning the reproductive and productive performance of large herds of dairy cows in Iran, including group 1) milk urea nitrogen (MUN) less than 12.96 mg/dl; group 2) MUN between 12.96 and 14.35 mg/dl; group 3) MUN between 14.35 and 15.88 mg/dl; and group 4) MUN over 15.88 mg/dl. Initially, a list of each dairy herd involved in Iran's DHI program was prepared, and 30 dairy herds with an excess of 1000 lactating cows were randomly selected. In order to mix the data on reproduction and milk composition, FoxPro 2.6 software was used. A statistical analysis of milk urea nitrogen and other independent variables was conducted through analysis of variance. The aforementioned results regarding the importance of reproduction and pregnancy rate in lactating cows and determining the appropriate concentration of milk urea nitrogen indicate that this parameter could be optimally used to control the protein-to-energy ratio and protein consumption of lactating cows in their diet.

***Keywords*:** milk urea nitrogen (MUN); reproductive performance; fertility; Holstein

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INTRODUCTION

Although having adequate knowledge regarding factors impacting reproductive performance in dairy herds to manage reproduction effectively is imperative, such strategies are better harnessed when coupled with good management and nutritional strategies. As an example, performing higher concentration of protein in the diet of dairy cows will result in some damage to the performance such as a slight decrease in uterine pH, changes in uterine secretions, reduced embryo survival, and impaired embryonic development (Rajala-Schultz et al., 2001; Guo et al., 2004; Hojman et al., 2004). The mean of liver in order to detoxification of performed ammonia in the systemic circulation is production of urea, which is subsequently diffuses or is transported to other fluid pools in the body such as milk in the mammary gland and liquid in the rumen. The urea found in milk, called milk urea nitrogen (MUN), is correlated to BUN (Ruska & Junkens, 2014). The main dietary factors, including CP and energy percentages, affect the amount of urea in blood, plasma, urine, and milk (Roseler et al., 1993), indicating a clear positive correlation between urea and energy balance and possibly with the energy concentration of the milk (DePeters and Ferguson, 1992b; Ciszuk & Gebregziabher 1994; Burgos et al. 2010). Monitoring Milk urea nitrogen (MUN) is a useful means for assessing protein nutrition and protein: energy synchronization in dairy cows (Butler et al., 1996; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001). Furthermore, the index is convenient and noninvasive and may be practical as a management tool to improve production efficiency (Godden et al., 2001a, b; Ayaşan et al., 2011). Existing hypotheses indicate that the higher MUN concentrations will impair either fertilization or embryonic development through either a direct effect of altering the uterine environment (Godden et al., 2001a, b; Garcia-Bojalil et al., 1994) or through an indirect influence on energy status (Godden et al., 2001a,b; Broderick and Clayton, 1997). Nonetheless, there are various recommendations for MUN concentration. For instance, according to Butler et al. (1996), it has been shown that a reduction of about 20% in conception rate in dairy cows will result in an MUN concentration higher than 19 mg/dL. On the other hand, surprisingly, Rajala-Schultz et al. (2001) suggested that an even lower MUN concentration (>15.4 mg/dL) is inversely associated with the rate of fertility in dairy cows. Yet, there is scarce information on the definite MUN concentration at which substantial impairment of reproductive performance occurs. In this case, various fertility parameters, such as days open, also known as calving to conception interval, can easily be determined, which and is an appropriate in monitoring reproductive efficiency. All things considered, however, it is not clear how farmers should use this information or which MUN value is more associated with days open, i.e., if farmers are supposed to use the first MUN analysis after calving, the highest MUN value obtained from calving to conception, or the average MUN analyzed during days open. Thus, this study was performed with the main objective of evaluating the association of monthly MUN concentrations (first, mean, or maximum MUN) before conception and days open in high-producing dairy cows from commercial herds in Iran.

MATERIALS AND METHODS

Data extraction and population of interest

The data regarding production and reproduction performance of dairy cow herds in Iran were obtained from the breeding center of animal science. The list of all the dairy herds participating in Iran's DHI program was prepared at the beginning of the research, and 30 herds with a capacity of more than 1000 lactating dairy cows were randomly selected as representatives of Iran's herds. To measure the data of these herds, the livestock breeding center was referred.

Criteria of Planning

For this purpose, milk records, including MUN and reproduction parameters such as the number of inseminations, the number of births, and the number of were used.

Experimental groups

Mean monthly MUN levels were calculated for each cow during the pre-pregnancy and post-pregnancy periods and divided into four groups.

Parameters

They included insemination per pregnancy, conception rate (fertility percentage), days to first insemination, open days, abortion rate and calving interval.

Statistical model and analyzing of data

Firstly, FoxPro 2.6 software was used to combine the reproductive data (taken from the country's livestock breeding center) with the milk composition data (Alborz milk analysis). SAS software was used for statistical analysis of the data. To analyze the descriptive statistics of the collected data related to the clinical and demographic characteristics of the cows under investigation, ratios, averages and standard deviations were calculated. As binary variables, pregnancy occurrence and non-pregnancy occurrence were scaled as "0" or "1" based on the presence or absence of each condition. A GLM procedure of SAS software (version 9.1) was used to determine the relationship between MUN concentration and births (1, 2 and more). Furthermore, the T-test was used to compare the different ranges of MUN.

RESULTS AND DISCUSSION

Milk urea nitrogen

Figure (1) illustrates the average concentration of milk urea nitrogen. The figure shows that milk urea nitrogen concentrations ranged between 12.15 and 17.50 mg/dl. The highest and lowest concentrations of milk urea nitrogen were related to the 26th herd and the 7th herd, respectively. The average concentration of milk urea nitrogen in pregnant and non-pregnant cows is given in Table (1). During the autumn and winter seasons, both pregnancy and non-pregnancy conditions have lower levels of MUN. Based on Table (1), pregnant cows have a lower average concentration of milk urea nitrogen than non-pregnant cows, but this difference is not statistically significant (P=0.089). In the first several weeks after calving, multiparous cows (MP) have a negative energy balance more easily than primiparous cows (PP) because of insufficient feed intake and high milk production, which may lead to reduced reproductive activities (Gross et al., 2015). According to Jonker et al. (1998), there was a decline in MUN concentration as DIM progressed starting from the second month, which resulted in a similar curve for both MUN and milk yield. In this line, MUN could be augmented as a result of negative energy balance, which a common problem during the period of early lactation (Jorritsma et al., 2003). Additionally, Spicer et al. (2000) have demonstrated that MUN will grow and remain constant, respectively, throughout the first three weeks of lactation and the remaining time. This trend in MUN can be attributed to a number of variables, such as physiological changes and the declining metabolic needs of lactation (Ayaşan et al., 2011). Conversely, lactating cows with positive energy balance perform better reproductively than cows with negative energy balance. In the absence of a negative energy balance, milk urea nitrogen can be used to assess nutritional status and reproductive performance (Raboisson et al., 2017). There may also be environmental factors which contribute to the partial increase in milk urea nitrogen in young cows and heifers with high production, such as stress caused by childbirth and physiological-hormonal changes.

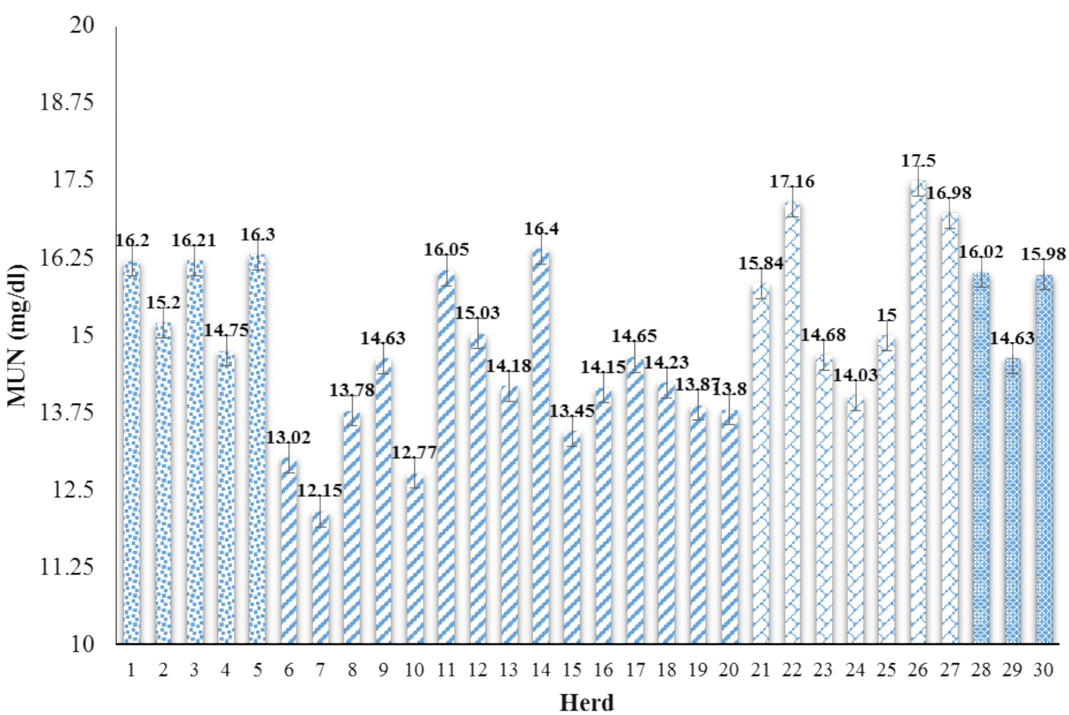

Figure (1) Average concentration of MUN in selected herds

Table 1. Results of logistic regression of selected dairy cows from 2019 to 2022

variable	Odds Ratio	Estimate ± SE	(95%) Confidence interval	P-Value
MUN (mg/dl)				
MUN ≥ 12.96	1	-	-	<0.05
14.38< MUN< 12.96	2.81	0.13 ± 1.1	0.1 - 76.55	-
15.88 < MUN< 14.38	1.64	0.08 ± 0.76	0.0 - 63.90	-
MUN ≥ 15.88	1.27	0.12 ± 1.05	0.1 - 96.16	-
Parity				
1	1	1	-	<0.001
≤ 2	3.22	0.09 ± 0.20	0.0 - 16.25	-
calving season				
1	1	-	-	< 0.05
2	1.02	0.09 ± 0.19	0.0 - 14.22	-
3	1.36	0.07 ± 0.35	0.0 - 26.44	-
4	1.68	0.12 ± 0.69	0.0 - 51.88	-
Reproductive season				
1	1	-	-	< 0.001
2	1.02	0.09 ± 0.26	0.0 - 14.23	-
3	2.93	0.12 ± 0.67	0.0 - 60.76	-
4	3.18	0.66 ± 0.91	0.0 - 14.79	-

1. 1) Spring; 2) Summer; 3) Autumn; 4) Winter

The possibility of abortion rate (OR) of multiparous (MP) cows increased (OR=2.95). The probability of abortion of cows that gave birth in the summer compared to cows that gave birth in the spring increased (OR=2.11). In general, cows that calve during the summer months are at a higher risk of abortion compared to cows that calve in the winter months (OR=2.11; CI%=0.56-0.95; and P<0.001). Two-way interaction between independent variables showed that the interaction between MUN and breeding season was statistically significant (P≥0.01). The results obtained in this study are consistent with other researchers' reports (Butler et al., 1996; Rangel et al., 2013; Munyaneza et al., 2017; Anand et al., 2019; SM et al., 2021). In the mentioned reports, there has been a remarkable negative relationship between concentration of BUN (>19 mg/dl) and the probability of pregnancy in cows. In accordance with our study, it has been found that changes of seasons and consequently fluctuation in weather condition would be affect the fertility and reproductive characteristics of dairy cows (Rajala-Schultz et al., 2001; Santos et al., 2009). These effects are mostly linked to seasonal variations in photoperiodic stimulation (Dahl et al., 2000) and fed ration quality (Rhodes et al., 2003). Furthermore, dairy producers frequently control the reproductive cycle of their herd such that calves occur during the colder months of the year in order to maximize the rate, as heat stress is more common from the end of spring to the beginning of fall. In related to the effects of seasonal variations, it has been reported that cows who calve in the spring and winter, are more likely to undergo higher risks of anestrus (Walsh et al., 2007) or postponed postpartum ovulation (Opsomer et al., 2000). Altogether, these are potential explanations for the greatest period of days open observed in those cows that calved in spring. Besides, Godden et al. (2001) report a non-linear negative relationship between milk urea nitrogen and fertility, so that cows with a milk urea nitrogen concentration of 12.6 to 18.2 mg/dl have a lower fertility rate.

Fertility characteristics

Figure (2) illustrates the fertility rate in 4 categories of milk urea nitrogen. As seen in Figure (2), high milk urea nitrogen leads to a lower fertility rate. For instance, high MUN (14.38 mg/dl) compared to low MUN (12.96-38 mg/dl) has significantly reduced the fertility rate (P=0.033). In line with the study results, data indicate that there is a remarkable negative relationship between MUN and fertility rate (Butler et al., 1996; Fergousen et al., 1993; Rangel et al., 2013; Munyaneza et al., 2017). In a meta-analytical study, it was stated that MUN more than 19 mg/dl (Butler et al., 1996) or more than 15.4 mg/dl (Rajala-Schultz et al., 2001) in comparison with lower MUN (Raboisson et al., 2017) has significantly decreased the chances of cows being pregnant. Furthermore, Jannah et al.,

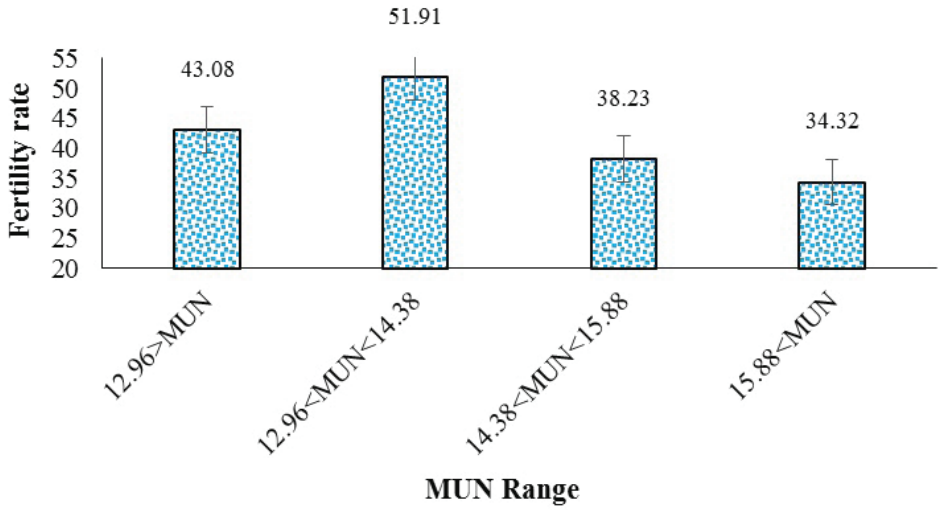

Figure (2) Fertility rate in 4 categories of MUN

(2019) reported a non-linear negative relationship between milk urea nitrogen and fertility. Also, Kananub et al., (2020) found that cows with medium levels of MUN (12.6 to 14 mg/dl) had a higher fertility rate compared to cows with high concentrations of MUN. In general, it has been found that excessive consumption of nitrogen increases the amount of ammonia, urea and other nitrogen compounds in the uterus and blood. These factors affect reproductive efficiency by delaying pregnancy, increasing the number of inseminations per pregnancy and fetal death (Fergousen et al., 1993; Butler et al., 1998; Rangel et al., 2013; Karapetkovska- Hristova et al., 2014; Munyaneza et al., 2017; Bobbo et al., 2020), which were in line with the results of the present study.

Given the strong relationship between MUN and plasma urea nitrogen (PUN), elevated PUN levels may have an impact on the reproductive system. Accordingly, Rhoads et al. (2006) revealed that embryo viability of lactating dairy cows decreased as a consequence of greater levels of PUN. The primary physiological explanation for this effect is that high PUN somewhat modifies the uterine pH (Elrod and Butler, 1993; Elrod et al., 1993; Rhoads et al., 2004). Being a small molecule, urea is capable of diffusing cell membranes of the uterus and reducing the pH level (Rhoads et al., 2004), eventually impairing embryo development (Ocon and Hansen, 2003). Also, it has reported that other alterations, such as variations in urea concentration, Mg, K, P, and Zn in uterine fluid may also be significant determinants (Jordan et al., 1983). Another theory is that excessive urea in the animal organism is a consequence of extensive liver metabolism to detoxify ammonia absorbed from the rumen, which might worsen the negative energy balance following calving. As a result, there may be irregular cycles and lower reproductive indices (Leroy et al., 2008).

Results of reproductive performances, including days to first insemination (DFS), conception rate (CR), insemination per pregnancy and days open are shown in Table (2). As can be seen, except for days before the first insemination, there is a significant difference in the area of mentioned characteristics between treatments (P>0.05). Treatment 4 had the biggest amount related to insemination to pregnancy, days open, and conception rate and the lowest for CR. With CR, however, the highest number has been achieved for treatment 2. It was determined that the high concentration of milk urea nitrogen is somehow equal to having a high interval between calving and first service (78.61 versus 67.13 days). Furthermore,

Table 2. Reproductive characteristics of dairy cows from 2019 to 2022

variable	MUN (mg/dl)					
	1	2	3	4	SEM	P-VALUE
Days to first insemination, d	67.13	69.71	72.35	78.61	11.14	0.78
Conception rate, %	43.08ab	51.91a	38.23bc	34.32c	2.23	0.03
Insemination per pregnancy	1.66bc	1.28c	1.78b	2.11a	0.06	0.04
Open days, d	109.20b	125.03b	132.91b	145.44a	13.10	0.05

Table 3. Correlation coefficient of reproductive characteristics with MUN

Variable							
MUN	-						
Days to first insemination, d	0.423	-					
Pregnancy rate, %	- 0.341	-0.421	-				
Insemination per pregnancy	0.375	0.215	- 0.475	-			
Open days, d	0.374	0.364	- 0.321	0.298	-		
Prior to 120 days of DIM, d	-0.564	- 0.364	0.468	- 0.472	- 0.399	-	
Calving Interval, d	0.219	0.395	- 0.456	0. 347	0.562	- 0.426	-

there is a significant negative relationship between MUN and conception rate (CR).

What could be concluded from our study is that being inversely affected by measured reproductive parameters by MUN concentrates. The results of the present study are similar to the results obtained from other studies (Doska et al., 2012; Rangel et al., 2013; Raboisson et al., 2017; Jannah et al., 2019; Braga Paiano et al., 2019; Kananub et al., 2020; Souza et al., 2021). Nonetheless, in another study regarding the effect of different levels of MUN on fertility in dairy herds, researchers found that the influence of MUN on reproductive performance may be affected by seasonal changes (Rangel et al., 2013). However, the results of some other studies have indicated that high levels of milk urea nitrogen do not affect the reproductive performance of high-producing cows (Pazzola et al., 2011). In general, it has been found that excessive consumption of nitrogen increases the amount of ammonia, urea and other nitrogen compounds in the uterus and blood. These factors influence reproductive efficiency by delaying pregnancy, increasing the number of inseminations per pregnancy and fetal death (Fergousen et al., 1993; Butler et al., 1998; Rangel et al., 2013; Karapetkovska-Hristova et al., 2014; Munyaneza et al., 2017; Bobbo et al., 2020), which were in line with the results of the present study.

Correlation of reproductive characteristics with milk urea nitrogen

In Table (3), the correlation results between reproductive characteristics and MUN are presented. What stands out from the table is that, there is a positive and significant correlation between the two MUN traits and the days to first insemination and open days (r=0.423+; $P<0.05$), and regarding the pregnancy rate and prior to 120 days of DIM there is a negative and significant correlation. However, there were no significant values between insemination ratio per pregnancy (r=0.375) and calving interval (r=0.219) ($P<0.05$). Various MUN values that impact fertility have been documented in the literature over time. For instance, Butler et al. (1996) found that dietary protein had a negative impact on fertility in dairy cows, with a MUN content exceeding 19 mg/dL. However, using cows sorted into quartiles for milk urea, Rajala-Schultz et al. (2001) assessed the influence of MUN on fertility in 1,249 dairy cows and found that MUN concentrations more than 15.4 mg/dL resulted in a reduced conception rate compared with lower MUN concentrations. Additionally, according to these investigators, cows with MUN levels below 10 mg/dL prior to conception had a 2.4-fold higher chance of becoming pregnant than cows with levels above 15.4 mg/dL. Recently, the relationship between MUN and some reproductive characteristics has been investigated by a group of researchers (Stoop et al. 2007; König et al. 2008; Mosaferi et al., 2011; Mucha & Strandberg 2011; Rzewuska & Strabel 2014). In this regard, Rzewuska and Strabel (2014) showed that animals with high production values and with medium and lower MUN concentrations have improved fertility traits. Similarly, Mitchell et al. (2005) showed lower MUN during the first and second lactations when examining fertility traits. All things considered, given the disparate findings, it is imperative to study the precise processes by which MUN concentration affects dairy cow fertility.

CONCLUSION

Based on the concentration of MUN in the selected herds according to the seasons, the highest and lowest concentrations of MUN occurred in spring and winter, respectively (MUN range 15.9-16.9 mg/dl and 14.9-2.15 mg/dl). Furthermore, this study found a direct relationship between the parity of the cow and the MUN concentration, so multiparous cows had a higher MUN value (15.88 mg/dl) than primiparous cows.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

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