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Interrelationship between the milk urea nitrogen level and milk coagulation traits in Holstein-Friesian cows with reproductive disorders in R. Macedonia

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Abstract: The goal of present study is to evaluate some possible effects of the high Milk Urea Nitrogen (MUN) content over the coagulation characteristics of the chosen milk in one dairy farm at R. Macedonia. Therefore, for this task we have studied various analysis of raw milk and technological properties such as: (active acidity - pH, titratable acidity - ^{0}SH, protein and fat content %, the time of initial coagulation and of total coagulation, pH and ^{0}SH of the whey as well the quantity of separated whey in %). In this experimental work, we have included a total of 149 Holstein Friesian cows with reproductive problems with an average service period of 150.92 ± 79.21 days. The samples of milk were divided according to the MUN level into two groups: the group N with n = 10 cows sample (milk urea nitrogen level < 6.50 mile-mol/L) and the group H with n = 15 cows sample (milk urea nitrogen level > 6.50 mile-mol/L).

The present surveys were conducted during the period March 2012- March 2013 in one dairy farm in R. Macedonia. The Means, LSD test and correlation coefficients for the investigated characteristics were calculated between the groups N and H dairy cows. And there were statistically significant differences between the average daily milk yield (DMY) (22.27, $p < 0.05$ for N group and 25.7, $p < 0.05$ for H group), MUN level (6.22, $p < 0.001$ for N group and 10.22, $p < 0.001$ for H group), Time of total coagulation (49.00 min. for N group and 58.26, $p < 0.001$ for H group) and the Separated quantity of whey (74.94% $p < 0.001$ for N group and 84.90, $p < 0.001$ for H group).

Keywords: Milk urea nitrogen, technological properties, Holstein Friesian breed, reproductive disorders

Introduction

Physiologically, in ruminants, excess ammonia produced from microbial degradation of dietary protein in the rumen is absorbed through the rumen wall, and carried to the liver where it is converted to urea. Urea carried in the blood also equilibrates rapidly between other body fluids and is therefore a common constituent of all of them, including milk as a consequence of the free diffusion of the urea molecule throughout the mammary epithelium. Even though the heritability of milk urea nitrogen (MUN) in dairy cows was shown to vary between 0.15 and 0.22 (Mitchell et al., 2005) [1], and also the MUN is shown to be influenced by environmental factors, such as parity, season, stage of lactation, milk yield and herd (Giaccone et al., 2007) [2], the major determinants of urea formation are the nutritional factors (Arunvipas et al., 2003) [3], mainly due to the amount of daily crude protein (CP) intake and the dietary ratio of CP to energy intakes. In some other studies, the milk urea levels were significantly influenced by dietary protein levels or degradability (Moharrery, 2004 and Zhai et al. 2006) [4-5], but It was not found much effective in some other studies (Davidson et al., 2003) [6] and (Flis and Wattiaux, 2005) [7]. Further for example, Carlsson and Pehrson (1994) [8], were estimated that milk urea concentration increased by between 12 - 18 mg/L for each additional 60 g of digestible crude protein fed to cows already receiving adequate protein.

The considerable interest has developed in using MUN as a monitor of the efficiency of nitrogen utilization by dairy cows (Baker and Ferguson, 1995) [9]. An overfeeding protein with a high urea concentration has been shown to have a

negative impact on health and fertility in dairy cattle. At the present time, there is also uncertainty as to whether the elevated urea concentrations are directly responsible for decreasing conception rates, possibly due to an associated decrease in pH in the uterine lumen (Rhoads et al. 2004) [10].

According to Janů et al. (2007) [11] and Hanus et al. (2010) [12], the technological quality of milk is influenced not only by fat, protein, lactose and solids non-fat but also by such parameters as active acidity and titratable acidity. Besides, rennet coagulation time (RCT), firmness of rennet curd, and volume of whey separated out during the process of enzymatic coagulation of milk are also very important (Hanus et al., 2007) [13]. The RCT of milk influences significantly the process of cheesemaking and also cheese yield, and its quality (Johnson et al., 2001) [14]. It is also an important parameter when evaluating milk suitability for processing and cheesemaking (Cassandro et al., 2008) [15]. Many studies have shown that high concentrations of MUN have negative effects on cheese making processes.

In particular, high concentrations of urea are the direct or indirect cause of numerous problems, such as an increase in coagulation time, the formation of a more fragile and less structured curd, premature development of irregular fermentations, and a more intense proteolysis. With the analysis of MUN, it is possible to avoid possible problems in the cheese making processes. Thus we are able to obtain information on possible trends and the results of genetic improvement programs of dairy herds in terms of total quality of milk and its acceptability as raw material for the milk food chain.

Materials and Method Used

a. Dairy herd management

On the farm has been growing (bred) 340 dairy cows and 230 heifers from a Holstein Frisian breed. Dairy cows in the herd have average lactation milk yield from 4800 -5500 kg. with 3.86% milk fat. The obtained milk is purchased by milk processing establishments and depending on the quality and the degree of acidity is processed into yogurt, soft and hard cheeses. In the cows rearing are involved 28 workers, 2 veterinarians. The work on the farm is organized in two shifts - from 6.00 to 21.00 o'clock and from 21.00 - 06.00 hours. At the time of milk sampling, the farm workers were investigated to determine the ration compositions, afterward, the composition of distributed rations were noted a representative sample of this ration is taken for the analysis. Information's about lactation row and calving dates were obtained from the breeding office.

b. Dairy cows feeding

The cows were kept in the free-boxing system with milking hall type "herringbone" pattern De Laval. The cows were milked twice a day between 06.00/07.00 and 17.00/18.00 daily regime (the cows up to 150 days after calving, were milked three times).

Based on average daily milk yield, feeding was allocated to the following groups:

•First Group:- fresh calved cows and heifers with a daily milk yield 30-32 kg of milk.

•Second Group:- fresh calved cows and heifers with a daily milk yield 22-25 kg of milk.

•Third Group:- The cows and heifers with a daily milk yield 15-24 kg of milk.

•Fourth Group:- The cows before drying.

•Fifth Group:- The dry cows.

For the present experimental study, we selected only the cows from the group first to third. As for the diet of dairy cows on the dairy farm feed was given throughout the year as a total mixed ration, and the cows were never turned out to graze. Diets feed before and after calving were formulated to exceed National Research Council recommendations (NRC, 2001) [16], and the residues of the dietary feed were generally observed in the herd.The rations were homogenized in a specialized trailer- Mixer and were set after the morning milking, as being available to the animals for 24 hours.

The cows had constant access to mineral-vitamin blocks and water for drinking. The values of diet compositions and nutrient intakes have been shown in Table 1. There in Table 1, the measured chemical composition of the feed included in the ration of experimental cows in percentage (%) values. Some other relevant details in out the used experimental techniques have been given our recently published articles [17-19].

Table 1. Description of data used as respects diet composition and nutrient intakes at experimental cows with a live weight 600-620 kg and average annual lactation 6500-7500 kg.

Composition of the	(I) Phase of the lactation (100 days) and the daily milk quantity is 30-32 kg		(II) Phase of the lactation (100-200 days) and the daily milk quantity is 22-25 kg		(III) Phase of the lactation (200 – 305 days) and the daily milk quality is 13-15 kg	
Hay, in kg.	4.2	4.5				
Concentrate mixture, in kg.	8.6	9.95	6.1	6.7	4.5	4.95
Straw, in kg.	0.15	0.30	0.6	1.0	2.2	2.5
Sugar beet pulp, in kg.	1.0	0.7	1.0	0.7	---	---
Haylage, in kg.	6.0	7	5	6	4	5
Silage, in kg	21.5	20	18.5	20	16.5	17.5
Provided DM/kg	20.5	21.5	18.3	18.75	15.2	15.6
Crude protein, in (gram)	3240	3440	2580	2740	1870	2210
Digestible crude protein (gram)	2430	2250	1820	1640	1160	1480
Percentage of protein, in (kg/SM)	16	16.5	14.2	14.8	12.8	14.1
Total energy.	140	144	118	114	89	94
NEL (MJ/кг DM)	6.85	6.65	6.45	6.19	6.20	5.85
CF, in %	16.8	17.2	18.5	19.6	21	24
ADF, in %	22	21.7	24	25	26	27
NDF, in %	37	36	42	43	45	46
Ca, in %	0.85	0.85	0.70	0.75	0.65	0.68
P, in %	0.52	0.50	0.42	0.42	0.38	0.36
NaCl, in %	0.50	0.30	0.45	0.30	0.40	0.25

c. Organization of the reproductive process

The farm produces artificial insemination of cows and heifers after oestrus detection and determination of the optimal time. In order to have higher results with respect to the fertility in cows and heifers needed proper registration of animals found in the "standing phase" of estrus. It has been proceed by the fact that 70% of animals showing "standing phase" in the time interval between 18-00 hours (evening) and at 6-00 hours (morning). Each cow has been marked with veterinary ear tag that is visible at least it three meters. If the workers are not confident about recognizing the animal and/or to record his earmark that is marked on the plate with the name and details of the animal. Recognizing the signs of oestrus is an important point in the detection of the animals. Therefore, the workers conducted preliminary training for different phases of the sexual cycle. For artificial insemination is used a cryopreserved semen is imported from the companies Swissgenetics for the Semex and Alta genetics. The clinical studies of cows were performed by veterinarians on the farm at R. Macedonia and the animals were registered with reproductive disorders such as: anoestrus, nymphomania, hypofunction of the ovaries, ovarian cysts and the presence of persisting corpus luteum.

d. The examination for problematic cows

To recognize the problematic cows or cows suffering from some diseases due to health problem, we performed the ultrasound examination of such cows and this has been completed by using the ultrasound scanner Aloka 500 owned by the farm with 5MHz linear transducer.
The implementation of the examination is carried out in following order:

- The cow is examined in place of her bedside, by fixing of two assistants /worker from the farm.
- The probe is placed in pre-cleaned of the feces rectum.
- The transducer is directed sequentially to the left and right ovary thus allowing intimate contact with the corresponding surface of the ovary
- Has been performed circular movements until the screen shows an image of a section through the uterine horns, bladder and ovaries.
- Based on observed ovarian formations (formations with a different optical density with size more than 2 cm) is diagnosed.

Definitive diagnoses have been considered on the basis of rectal examination and ultrasound. A pie - chart of reproductive disorders has been shown in Figure 1. The distribution of reproductive disorders at our dairy farm is in correlations with some other workers in the field of Veterinary Science [20].

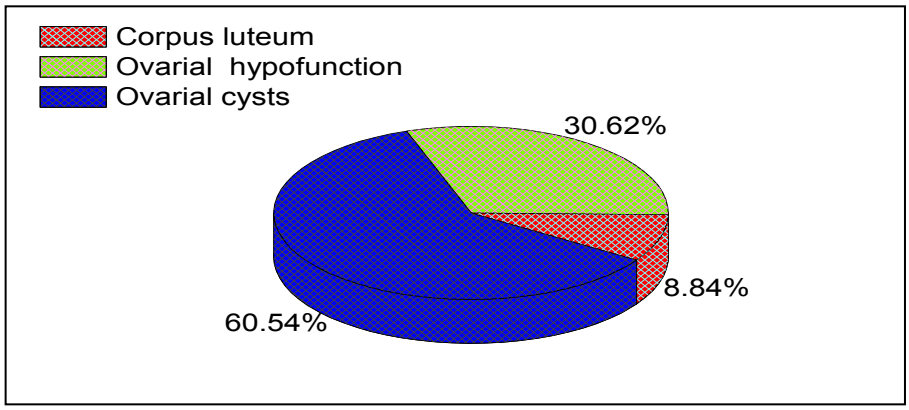

Figure 1: The distribution of reproductive disorders of cows in the dairy farm.

e. The sampling procedure

The present study was carried out during the period from March 2012- March 2013. Data were obtained by randomly collecting 90 samples of milk from cows with reproductive disorders from one dairy farm in R Macedonia. The milk samples were collected aseptically during the routine morning milking in sterilized plastic cups (50ml), which were after that kept in ice and immediately transported to the laboratory. The milk analyses were done in the "Bitola laboratory"in R. Macedonia.

f. Milk analysis

The milk yield was monitored individually of 24 hour period, during the morning and evening milking. Experimental samples of milk were taken of the morning milking. They were shipped in an insulated package to the laboratory and were analyzed within 3 hours by following reception; fat, protein and lactose contents and were assessed using a Milkoscan instrument (Foss Electric - Hillerod, Denmark). The milk urea nitrogen (MUN) (mile-mol/L) level was determined by using a Photometric method for the determination of urea in milk (Merck KGaA - 64271 Darmstadt, Germany) and also by spectrophotometry (Pharo 300 Spectroquant Merck. Germany). The pH was measured by potentiometer using a pH meter by SCHOTT Lab 860 from Germany. The somatic cell count was performed with the help of a Somaskop named Alfa Laval from Germany. For the Rennet coagulation time (in minutes) was used manual measurement as it is used by other workers (Storry et al. 1983) [21] for this the freshly prepared1:10 diluted chymosin (*Chymosin Forte 1: 50 000*) was combined with milk at 0.03% (v/v) and incubated at 30 °C. The time from the addition of diluted chymosin to the first sign of sudden breakdown of the film on the tube wall was measured and defined as the RCT.

g. Milk coagulation process

One hour after milking, milk from the selected experimental cows was transported in containers from inox-steel, pre-chilled at a temperature up to 8^0 C at the "Laboratory of dairy chemistry and technology", Faculty of biotechnical sciences, Bitola. In the double bottom stabilizer, 5 kg of milk from each selected cow was heated to the renneting temperature of 35^0C and was added rennet powder, according to the rules of the company-manufacturer. The mixture was mixed well and the clotting time T (min), which is the time period starting from the addition of rennet to the first appearance of clots of milk solution, was recorded. Normal coagulation was reported for a period of 45-60 minutes. Primarily, it was determined the time for the initial coagulation, and then the time of total coagulation. After cutting the coagulum was determined pH, SH ^{0}, and the percentage of the separated whey (syneresis) in (%).The present chemical analyses and coagulation process of milk have been performed in accordance to our earlier publications (Vesna K. Hristova et. al., 2014) [17-19].

Results and Discussions

a. Means / standard deviation (S.D.) and coefficient of variation

The statistics description of the present experimental work along with traits are presented in Table 2. Means (standard deviation) of average daily milk yield (L/d), Milk urea nitrogen level (mile-mol/L) and (mg/dl), protein content (%), fat

content (%), SNF (%), lactose (%), SCC (No/ml), pH and ^{0}SH of raw milk for group N were 22.27 (3.33), 6.22 (1.40) and 14.31 (3.23), 3.18 (0.09), 3.77 (0.07), 8.33 (0.07), 4.45 (0.15), 223 333.3 (8146.96), 6.66 (0.11) and 8.26 (0.82) respectively. The mean (standard deviation) of clotting properties of the milk from group N, the Time of initial coagulation (min), Time of total coagulation (min), pH and ^{0}SH of whey as well as Separated quantity of whey (%) were 10.80 (1.87), 49.00 (4.94), 6.52 (0.08), 5.98 (0.82) and 74.94 (5.16). The statistical mean (standard deviation) of studied traits for group H, average daily milk yield (L/d), Milk urea nitrogen level (mmol/L) and (mg/dl), protein content (%), fat content (%), SNF (%), lactose (%), SCC (No/ml), pH and ^{0}SH of raw milk were 25.7 (5.46), 10.22 (1.73) and 23.50 (3.99), 3.42 (0.22), 3.89 (0.32), 8.48 (0.15), 4.40 (0.19), 225 000 (17837.65), 6.54 (0.06) and 8.24 (1.25).

Table 2. Descriptive statistics of daily milk yield, composition and clotting properties of the individual milk samples of Holstein cows (group N and group H).

Indicators	milk urea nitrogen level < 6.50 mile-mol/L(group N)			milk urea nitrogen level > 6.50 mile-mol/L(group H)		
	No.	Mean ± S. D.	min. - max	No.	Mean ± S. D.	min. - max
Average daily milk yield. liters	10	22.270±3..33 [B]	18.00 - 28.90	15	25.7 ±5.46 [B]	16.00 - 35.00
Milk urea nitrogen (MUN) conc.mmol/L	10	6.22 ± 1.40 [A]	3.1-7.9	15	10.22 ± 1.73 [A]	7.4-12.5
Milk urea nitrogen (MUN) conc. mg/dl	10	14.31 ± 3.23 [A]	7.1-18.2	15	23.50 ± 3.99 [A]	17.0-28.7
Protein. %	10	3.18 ± 0.09	3.1-3.4	15	3.42 ± 0.22	2.8-3.7
Fats %	10	3.77± 0.07	3.7-3.9	12	3.89 ± 0.32	3.4-4.8
Solids non fat (SNF) %	10	8.33±0.07	8.3-8.4	15	8.48±0.15	8.2-8.8
Lactose %	10	4.45±0.15	4.3-4.7	15	4.40 ± 0.19	4.1-4.8
Somatic cells count (SCC) No / ml	10	223 333.3±8146. 96	210000-230000	15	225 000.0±17837 .65	200000-250000
pH of raw milk	10	6.66±0.11	6.5-6.8	15	6.54±0.06	6.6-6.8
^{0}SH of raw milk	10	8.26 ± 0.82	7.2-9.8	15	8.24 ± 1.25	5.8-10.3
Time of initial coagulation. minutes.	10	10.80± 1.87	8.0-13.0	15	10.80 ±3.34	6.0-20.0
Time of total coagulation. minutes.	10	49.00±4.94 [A]	43.0-55.0	15	58.26±4.18 [A]	51.0 -65.0
pH of the way	10	6.52±0.08	6.4-6.6	15	6.60±0.08	6.0-6.7
^{0}SH of the way	10	5.98±0.82	4.5-7.4	15	6.14±0.97	4.8-8.6
Separated quantity of way %	10	74.94±5.16 [A]	68.30-83.00	15	84.90±7.99 [A]	70.00-94.00

Note:- [A]differences between the average values in rows are statistically significant at $p < 0.001$
[B]differences between the average values in rows are statistically significant at $p < 0.05$.

As far as the Time of initial coagulation (min), Time of total coagulation (min), pH and ^{0}SH of whey as well as Separated quantity of whey (%) for the group H, were 10.80 (3.34), 58.26 (4.18), 6.60 (0.08), 6.14 (0.97) and 84.90 (7.99) respectively. The outcomes from the analysis of variance, reported in Table 2, in groups N and H, milk urea nitrogen level, time of total coagulations and the quantity of separated whey were highly significant ($p < 0.001$) and average daily milk yield ($p < 0.05$). As expected, in our study the H group of Holstein dairy cows exhibited the highest average daily milk yield (25.7 kg/d) and lower milk quality; fat, protein, milk urea contents were 3.89%, 3.42%, 10.22 mile-mol/L and prolonged time of total coagulation (58.26 minute). Values of milk coagulation properties were slightly worse than those reported by Cassandro et al. (2008) [15] for Italian Holstein-Friesian cows, but similar to those reported by De Marchi et al. (2008) [22]. In terms of the results obtained about the average values of the % of separated quantity of whey, between the groups N and H (74.94, $p < 0.001$ and 84.90, $p < 0.001$) the significant differences have been confirmed. From this, one can see that the separation of whey at milk with high milk urea nitrogen level was higher, so the cheese yield will be lower due to the migration of different amounts of fat and casein in the whey. In the cheesemaking process high yields of cheese from each liter of milk are produced if clotting is rapid and if the curd riches high consistency (Chapman, 1981) [23]. This is because faster renneting time and greater curd consistencies (Storry et al. 1983) [21], correspond to greater concentration of milk components, particularly of casein.Since the consistency of the curd and separated whey in their study was measured with an Instrom Universal Testing Instrument, it is difficult to compare their extraordinary results with those in this paper.

b. Correlation effects

Further for to the better understanding and/or to unearth the basic contemporary need of the present dairy farm, we have studied the correlation coefficients between milk urea nitrogen (MUN) level and raw milk performances, milk coagulation properties and daily milk yield in cows with reproductive disorders, and it is depicted in Figures 1 - 3 and

also in Table 3 respectively. The figure 1, is for the correlation between MUN and the time of initial coagulation of milk in cows with reproductive disorders (group N), Figure 2, for the Correlation between MUN and separated way of milk coagulation process (group H) and the Figure 3, for the correlation between MUN and separated way of milk coagulation process (group N).

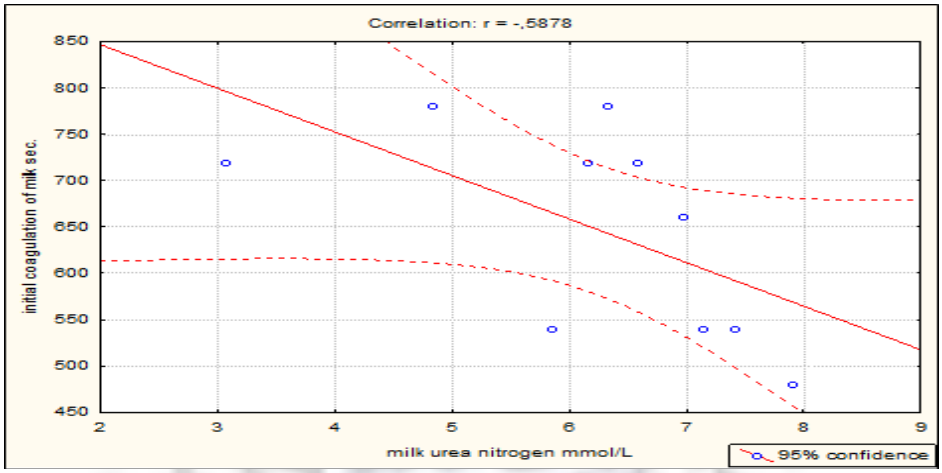

Figure 1: Correlation between MUN and time of initial coagulation of milk in cows with reproductive disorders (group N).

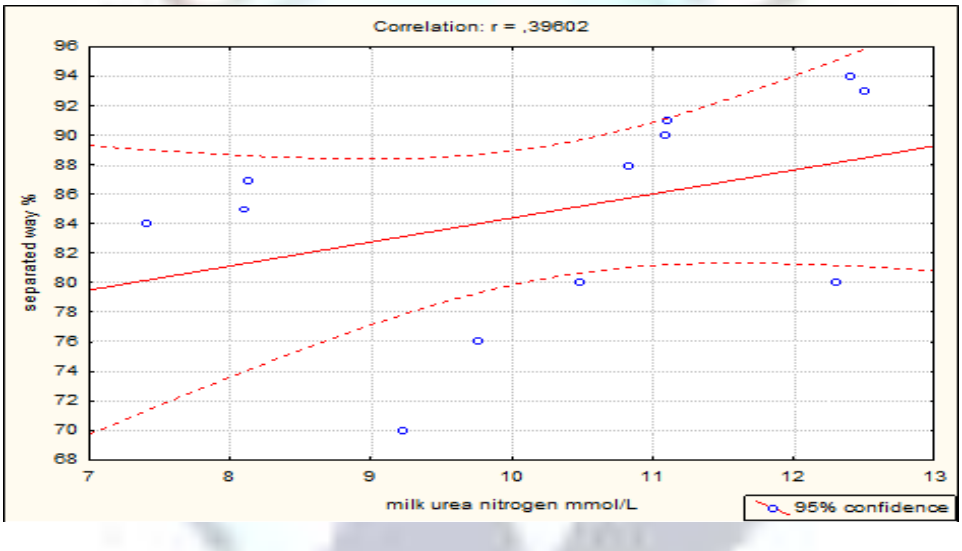

Figure 2: Correlation between MUN and separated way of milk coagulation process (group H).

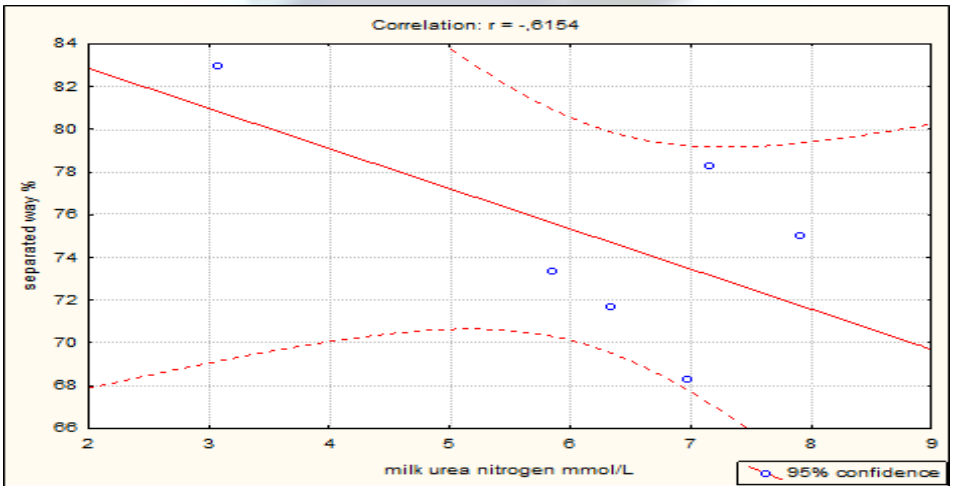

Figure 3: Correlation between MUN and separated quantity way of milk coagulation process (group N).

Based on the above all figures, we find the values of the correlation factor "r" were in such order: (i) For (group N) i.e. the correlation between MUN and the time of initial coagulation of milk in cows with reproductive disorders was (r = 0.5878), for (group H), i.e. the correlation between MUN and separated way of milk coagulation process was (r = 0.3960) and finally for the (group N) the correlation between MUN and separated way of milk coagulation process was (r = 0.6154) all these are results in a good agreement with the results obtained by some other works in the field of Dairy Sciences [24-26].

Table 3. Correlation coefficients between Milk urea nitrogen level and raw milk performances, milk coagulation properties and daily milk yield in cows with reproductive disorders.

N group	MUN (mmol/L)	Protein content (%)	Fat content (%)	Time of initial coagulation (min)	Time of total coagulation (min)	Separated whey (%)	Average daily milk yield
MUN (mmol/L)	**1**	**-0.89 ****	**0.63**	**-0.59**	**0**	**- 0.62**	**0.01**
H group	MUN (mmol/L)	Protein content (%)	Fat content (%)	Time of initial coagulation (min)	Time of total coagulation (min)	Separated whey (%)	Average daily milk yield
MUN (mmol/L)	**1**	**-0.94**	**0.75**	**-0.17**	**0.07**	**0.39**	**0.48**

Note:- In the above table, the usual meaning of *****p means < 0.05, ******p means < 0.01 and *******p means < 0.001

From Table 3, it is predicted that in group N and H, the relationships between milk urea nitrogen level and protein content exist a large negative correlation (r = - 0.89, and p < 0.01) and r = - 0.94 at milk with high urea nitrogen level. In accordance with our results, there was a negative association between milk urea and milk total protein content (Ferguson et al., 1997) [27]. The inverse relationship between milk urea and milk total protein pinpoints the alternative pathways that N can follow: incorporation into milk protein or excreted as urea. However, other studies (Sharma et al., 2009) [28] found no significant relationship between milk total protein and milk urea. In the group H, moderate correlation between urea content and milk yield was found to be (r = 0.48) and not significant as opposed to the literature (Rajala-Schultz et al 2003 and Roy et al.2004) [29-30]. In agreement with the results of the authors (Rajala-Schultz and Saville, 2003) [29], there was a consistent positive association between MUN and milk fat content of both groups N and H. A possible explanation for this association could be that high amount of NDF may increase the milk fat content and at the same time raises milk urea nitrogen level because of the high degradability of its protein. Other studies have reported no association between milk urea and either milk fat percentages (Klusmeyer et al., 1990) [31].

Part of the metabolic energy sources are regularly consumed for ammonia detoxification to urea in the liver. A moderate positive correlation between milk urea nitrogen and average daily milk yield at an H group of cows was found. The same results have been shown by Kucera (2003) [32], where a positive relationship and a certain stagnation of the milk yield was observed as for a very high level of urea, over 10 mile-mol·L^{-1}.

Conclusions or An Outlook

The present study has shown the existence of significant effect for milk urea nitrogen level over the milk coagulation traits in Holstein-Friesian cows with reproductive disorders in R. Macedonia and its outcomes are followings:

This study confirmed that high concentrations of urea are the direct or indirect cause of numerous problems, such as an increase in coagulation time, the formation of a more fragile and less structured curd and high % of separated whey. With the analysis of MUN, it is possible to avoid possible problems in the cheese making process, but further research would be required for the possible use of MUN assessment as a management tool for improving the reproductive disorder at dairy cows in R.Macedonia farms.The findings from the analyses of raw milk and such as: active acidity - pH, titratable acidity - ^{0}SH, protein and fat content %, the time of initial coagulation and of total coagulation, pH and ^{0}SH of the whey as well the quantity of separated whey in % are in good agreements with the other workers in the field of Dairy Industry. Our present experimental work, confirmed that high concentration of urea are the direct or indirect cause of numerous problems, such as increase in coagulation time, the formation of more fragile and less structured curd and high % of separated whey. Therefore, with the analysis of MUN, it is possible to avoid eventual problems in the cheese making process.

Also the further research would be required by using milk urea concentration testing it as a tool to the monitor reproductive performance in R. Macedonia dairy herds and it is concluded that the MUN testing may have utility as a monitoring or diagnostic tool for reproductive performance.

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