International Journal of Enhanced Research in Science Technology & Engineering, ISSN: 2319-7463
Vol. 3 Issue 3, March-2014, pp: (522-529), Impact Factor: 1.252, Available online at: www.erpublications.com

Study of coagulation properties of Holstein cow's milk depending on the level of milk urea nitrogen in Macedonia dairy farms

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Abstract: In this paper an attempt has been made to obtain some investigations for coagulation properties of milk produced by Holstein-Friesian cows reared on local Pelagonia's farms in the R. Macedonia. Based on an initial screening in a herd of 60 Holstein-Friesians, the twenty five individual Holstein-Friesian cows were selected for good and poor chymosin - induced coagulation properties. These 25 selected cows were followed and re-sampled on several occasions to evaluate possible changes in coagulation properties according to the milk urea nitrogen level. We formed two groups of cow's milk amongst 25 cows, group with normal milk urea nitrogen level, (< 6.2 mile-mol/L) and with high milk urea nitrogen level (> 6.2 mile-mol/L) . In the follow-up study, the milk samples were analyzed for average values, such as DMY, MUN- milk urea nitrogen, (mile-mol/L), lactose, protein and fat content (%), SNF- solids non fat (%) somatic cell count, pH, titratable acidity (Soxhlet-Henkel acidity, ^{0}SH), as well as the rennet coagulation properties. The study describes the action of rennet on Holstein -Friesian breed cow's milk with normal and high milk urea nitrogen level, as well as the effect of pH and temperature on the coagulum development. Finally, the present investigations were undertaken to obtain a better insight into the problem of rennet coagulation properties of Holstein-Friesian cow's milk, based on the level of milk urea nitrogen content.

Keywords: Holstein- Friesian breed, DMY, Milk Urea Nitrogen (MUN) level, rennet coagulation properties.

Introduction

Within the worldwide level, as the dairy industry starts to grow and cheese manufacturing begins to be done in larger production facilities, it became apparent that a more scientific and standardized method for improving of milk coagulation properties should be developed. The dairy industries are more and more interested in improving of milk coagulation properties (MCP), as a basic requirement of milk for cheese production (Summer et al., 2002) [1], as well as their affection of the cheese-making process efficiency (Aleandri *et al*., 1989; De Marchi *et al*., 2008; Wedholm *et al*., 2006) [2-4], and cheese yield and quality (O'Callaghan *et al*., 2000) [5]. The efficient manufacture of high-quality cheese consistently is a highly complex biotechnological process involving controlled destabilization and gelation of the milk protein, fermentation of the milk sugar lactose to lactic acid, dehydration of the gel to obtain cheese curd, and maturation of the curd to a ripened cheese with the desired quality attributes (sensory, aesthetic, usage, safety, convenience, wholesomeness, value for money) required by the consumers (O'Callaghan & Guinee, 2004) [6]. Therefore, the milk coagulation properties (MCP) might become very much beneficial at high levels, but there is a lack of support that it can be used in milk payment schemes as the accuracy of prediction models is not large enough for this purpose (De Marchi *et al*., 2009) [7].

The concentration and type of protein have a major influence on the micro-structure of acid- and rennet-coagulated milk gels, which markedly affects its rheological and syneretic properties, the recovery of fat and protein from milk to cheese and the yield of cheese (Schafer & Olson, 1975 [8]; Harwalkar & Kalab, 1980, 1981 [9, 10]; McMahon et al., 1993 [11]; Guinee et al., 1993 [12]). Besides these characteristics, genetic aspects play an important role in determining MCP. The present study about milk production potential is very limited. However, milk production varying between 1,800 and 12,775 kg during a lactation period between 9 and 18 months has been reported [1, 5 and references therein].

Numerous studies have demonstrated 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. [13-14].

The present investigations were undertaken to obtain a better insight into the problem of rennet coagulation characteristics of Holstein-Friesian breed cow's milk from Macedonia dairy farms. The study describes the action of rennet on cow's milk with different milk urea level, as well as the effect of pH and temperature on the coagulum development. Certainly, it would be of interest now to generalize these observations with the cheese technologies in dairy industries in R. Macedonia, and to verify if it is possible to correct the influence of milk urea by modifying cheese making parameters.

Materials and Methodology Used

A. Milk Samples Collections and Laboratory Analyses

Holstein-Friesian breed milk samples were taken at one dairy farm in the Bitola district in the Republic of Macedonia which is situated at an altitude of 1,233 meters above the Adriatic sea level. The dairy cows on the farm were fed ad libitum throughout the year as a total mixed ration, supplemented with concentrate according to standard practice and the cows were never turned out to graze. Rations before and after calving were formulated to exceed National Research Council recommendations (NRC, 2001) [15], and the residues of the dietary feed were generally observed in the herd. All cows received the same lactation diet for ad libitum intake throughout the experimental period post calving. The milk samples were collected from the morning milking of the dairy cows (6.00 - 7.00 hours). In accordance with the rules for milk sampling, the milk samples were manually taken from the individual collector of the milking De Laval system in with a special sterile plastic cups (50ml). Samples were transported to the laboratory by movable refrigerator and kept in at the same temperature < 10 °C during the determination of milk quality parameters. The analysis of MCP was carried out following M. Mele, R. Dal Zotto et al. (2009); [16] briefly, milk samples (10 mL) were heated to 35 °C and 200 µL of rennet was added to the milk.

B. Coagulation of Milk

Examination and coagulation of milk were performed within 3 hours 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 ^{0}C and was added rennet powder, according to the rules of the company-manufacturer. The mixture was mixed well and the clotting time T (min), 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 [17].

Primarily, it was determined the time for the initial coagulation, and then the total coagulation time, as well as the sensory characteristics of the coagulum. And after that by cutting the coagulum the pH and ^{0}SH values were determined and the quantity of the separated whey (syneresis) in (%).

C. Determination of Milk Coagulation Activity

The rennet was commercial powder Chymosin - Forte, with a strength of the enzyme 1 : 50 000. Rennet solution of 0.4% was prepared and an appropriate amount of this solution was taken to give a visually observed coagulation time of approximately 10 minutes in milk. For measurement of the milk coagulation time the following two steps were used.

(i) Effect of temperature

The milk samples were adjusted to pH 6.6 -6.8 by slow addition of 1 M HCI, placed in a water bath, and the coagulation time was measured at temperatures over the range at 30 $^{\circ}$C to 45 °C (the attempts were made at an interval of 5 minutes apart and have found that a temperature of 35 ^{0} C is the most well-clotting)

(ii) Effect of pH

All samples were equilibrated at 35 °C and the coagulation time determined at pH between 6.20 and 7.00.

D. Determination of Curd Quality

The principle: We evaluated the quality of curd after the incubation.

The Technique: The incubated milk has been used for this, after adding rennet one hour in thermostat (at temperature 35°C). The evaluated curd quality was in Petri.

The quality of curd: The quality of curd/whey and also its appearance and properties are given in following Table. 1.

Table 1: For the explanation of curd quality

Quality Classes / Types	Appearance and properties of curd/whey
I	The appearance of curds and whey The curd is very good, has sturdy shape. The coagulum is firm, the whey is well gone out with a typical yellow-green color,

	no trace of the palm of the hand when we are pressing on the coagulum.
II	The whey is well gone out and clear with yellow – greenish color. The curd is good, has a less sturdy shape.
III	The whey is no well gone out and has white – greenish color. The curd is bad, soft, doesn´t hold together. Coagulum is loose, there is little trace of the palm of the hand when we are pressing on the coagulum
IV	The whey is no well gone out and has milk white color. The curd is very bad, doesn´t hold together at all. Coagulum is loose, does NOT cut with a knife, there are traces on the palm of the hand when we are pressing on the coagulum
V	The whey has a milky white color. Indistinctly or no coagulation of casein.

Results and Discussions

We collected milk samples from the dairy farm and performed the statistical analysis in our laboratory by various steps, a nice view of laboratory / instrumental analysis has been shown in Figures 1 - 3. The initial coagulation was determined by the time, and it can be examined, by pressing the palm of our hand on the coagulum surface as it is in Figure 1. The cutting of the coagulum with a cheese Harf has been shown in Figure 2, and the processes of squeezing the way and measurement of its quantity in percentage (%) also has been depicted in Figure 3. All the procedure and results are in good agreements with the workers in the field of research in dairy science [18, 19].

Figure. 1: A laboratory view for the Coagulation processes.

We measured various parameters and processed statistically, those values are depicted in Table 2. In the table the total 25 cows, distributed into 2 groups of cows, (i) Group – N Number of cows = 10 samples means cows with normal milk urea level and (ii) Group – H Number of cows = 15 samples with high milk urea nitrogen level. Parameters of variation were ≥ 31.5% for each milk coagulation properties, whereas for the other chemical parameters, they were ≤ 31.5% (Table 2). Coagulation properties were slightly worse at milk with increased urea nitrogen level as observed by some writers (e.g. Ikonen et al. 1999a, Caroli A. et al. 1990) [20-21]. According to the visual evaluation of curd properties and quality classes of curd, (Table 1), the curd of cow's milk from group N was evaluated in class I, while the curd quality of cow's milk from group H is rated between the classes III –IV. Testing of milk clotting ability, curd characteristics and separation of the soluble phase, give a good enough estimate of the milk coagulation ability of the individual samples of one dairy herds.

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Figure. 2:Cutting the coagulum with cheese Harf. Figure. 3:Squeezing the way and measurement of its quantity in %

Table 2. Differences in DMY (daily milk yield), milk chemical components, and physical and technological properties between N cows with normal milk urea nitrogen level and H cows with high milk urea nitrogen level

Various Parameters		**Group - N**		**Group - H**	
Abbreviation	**Units**	**x ± sd**	**Range (min-max)**	**x ± sd**	**Range (min-max)**
DMY	Lit/Day	22.27 ± 3.33^{B}	18.0 - 28.9	25.7 ± 5.46^{B}	16.0 - 35.0
FAT	(%)	3.77 ± 0.07	3.7 - 3.9	3.89 ± 0.32	3.4 - 4.8
LAC	(%)	4.45 ± 0.15	4.3 - 4.7	4.40 ± 0.19	4.1 - 4.8
SNF	(%)	8.33 ± 0.07	8.3 - 8.4	8.48 ± 0.15	8.2 - 8.8
PC	(%)	3.18 ± 0.09	3.1 - 3.4	3.42 ± 0.22	2.8 - 3.7
MUN	(mol/L)	6.22 ± 1.40^{A}	3.1 - 7.9	10.22 ± 1.73^{A}	7.4 - 12.5
SCC	(10^3 * ml^{-1})	223.3 ± 8.15	210 - 230	225.0 ± 17.84	200 - 250
pH	---	6.66 ± 0.11	6.5 - 6.8	6.54 ± 0.06	6.6 - 6.8
SH$^{\circ}$	0.25 mol.L^{-1} with NaOH	8.26 ± 0.82	7.2 - 9.8	8.24 ± 1.25	5.8 - 10.3
RCT	(second)	10.80 ± 1.87	8.0 - 13.0	10.80 ± 3.34	6.0 - 20.0
TCT	(second)	2940.0 ± 296.6^{A}	2580 - 3300	3496 ± 250.9^{A}	3060 - 3900
W	(%)	74.94 ± 5.16^{A}	68.3 - 83.0	84.90 ± 7.99^{A}	70.0 - 94.0
pH(W)	---	6.52 ± 0.08	6.4 - 6.6	6.60 ± 0.08	6.0 - 6.7
SH$^{\circ}$(W)	0.25 mol.L^{-1} with NaOH	5.98 ± 0.82	4.5 - 7.4	6.14 ± 0.97	4.8 - 8.6

Note:- Some abbreviations of the above table are such as:- Statistical significance of the differences: ^{A} means:- differences between the average values in rows are statistically significant at $p < 0.001$ and ^{B} means:- differences between the average values in rows are statistically significant at $p < 0.05$,

x ± sd means arithmetic mean values of different parameters with standard deviation, DMY – daily milk yield, FAT – fat content, LAC – lactose, SNF – solids non fat, PC – protein content, SCC – somatic cell count, MUN – milk urea nitrogen, pH – acidity, ^{0}SH– titratable acidity, RCT- rennet coagulation (gelation) time, TCT – total coagulation time, W – separated quantity of whey, pH(W)- whey acidity; ^{0}SH (W) – whey titratable acidity.

From Table 2, we can say, that the average values of daily milk yield (DMY) in H-cows were higher by 13.35% than N-cows and this high difference was significant (P< 0.001) also depicted in Figure 4 (a). The studied high daily milk yield (DMY) parameter had statistically significant influence (P < 0.05 and P < 0.001) on such milk indicators; FAT, lactose, solids non fat, MUN, TCT and separated quantity of whey. The differences of the content of FAT, LAC and SNF between the N and H groups have been seen in Figures 4 (b-d). From these figures one can conclude that in all these parameters there is existing difference in values between groups, especially the values are higher at group H, but there isn't statistical significance between these parameters. Depending on the chemical composition of the milk in Pelagonia dairy farms, these parameters are in good correlations with the other workers [22-24].

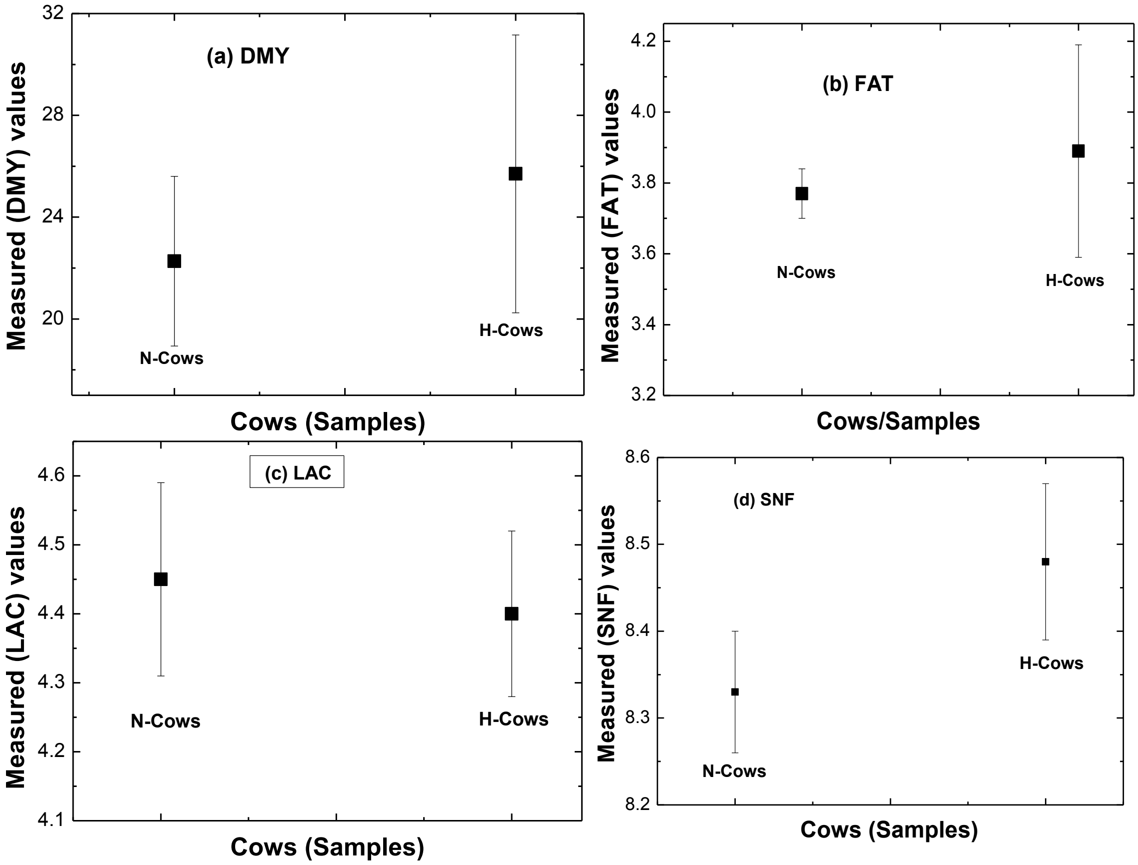
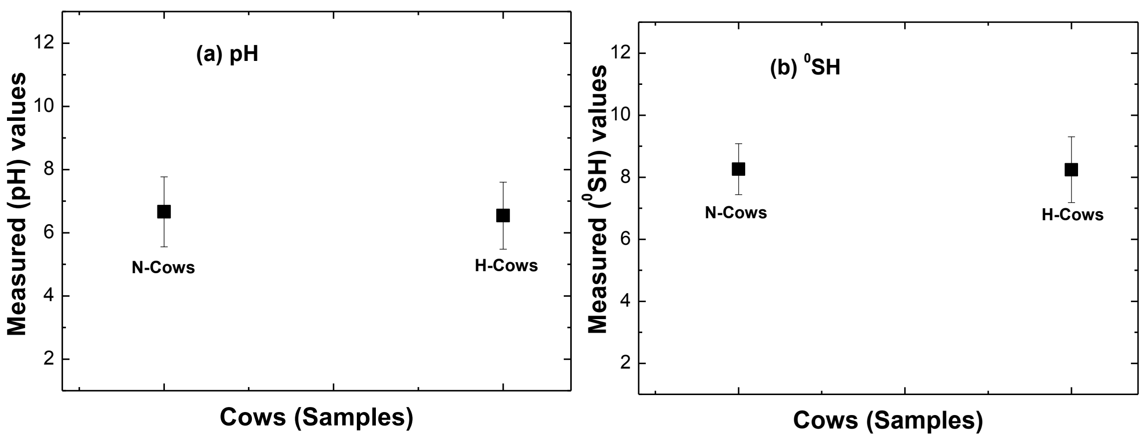


Figure 4 (a-d): For the statistically measured values of DMY, FAT, LAC and SNF for both groups of cows.

The pH values were also measured for N-cows and found an approximate 6.66 ± 0.11, and within the minimum and maximum range 6.5 - 6.8. Also the pH measured for H-cows and found approximate 6.54 ± 0.06, and within the minimum and maximum range 6.6 - 6.8. Therefore on behalf of measured pH values one can say that there is no fundamental effect on the milk coagulation properties in both groups of dairy cows. We have drawn the figures 5 (a-d) for the values of pH, $^{\circ}$SH, pH (W) and $^{\circ}$SH (W), from these figures one can see that these values are almost constant for N-cows and H-cows, although a slight difference between such values. Therefore behalf on these figures, we can say that, such parameters are in good correlations with the other workers [25-27] and the effect of these parameters pH, $^{\circ}$SH, pH (W) and $^{\circ}$SH (W), were of no practical relevance.



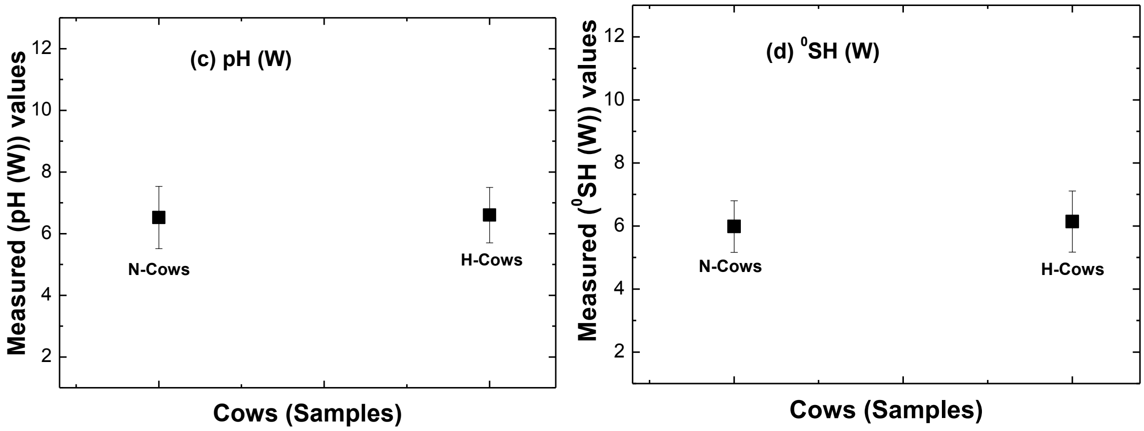


Figure 5 (a-d): For the statistically measured values of pH, $^{\circ}$SH, pH (W) and $^{\circ}$SH(W) for both groups of cows.

The milk urea is an energy and the nitrogen metabolism indicator [28], that is why milk urea nitrogen (MUN) was examined and it is also important for genetic [29] point of view. Its unsatisfactory (high, sometimes also low) values are often linked with aggravated reproductive performance [29, 30]. MUN was influenced significantly ($P \leq 0.05$) by high daily milk yield (DMY) in the present research work. In figures 6 (a-d), we have drawn some results from the study of MUN, RCT, TCT and W (in %). Also we can say that the values of RCT in Figure 6 (b) are almost constant for both the cows / samples, whereas the values of MUN, in figure 6 (a), TCT in figure 6 (c) and W (%) in figures 6 (d) are statistically significant. So, from these figures one can see that the values of TCT and W (in %) are strongly correlated for both the groups of N-cows and H-cows, where the prolonged total coagulation time –TCT, and higher percent of separated whey can be observed in milk in the H - group of cows, as a result of the effect of high milk urea nitrogen content. The present results are consistent with the findings obtained by other workers [25-30].

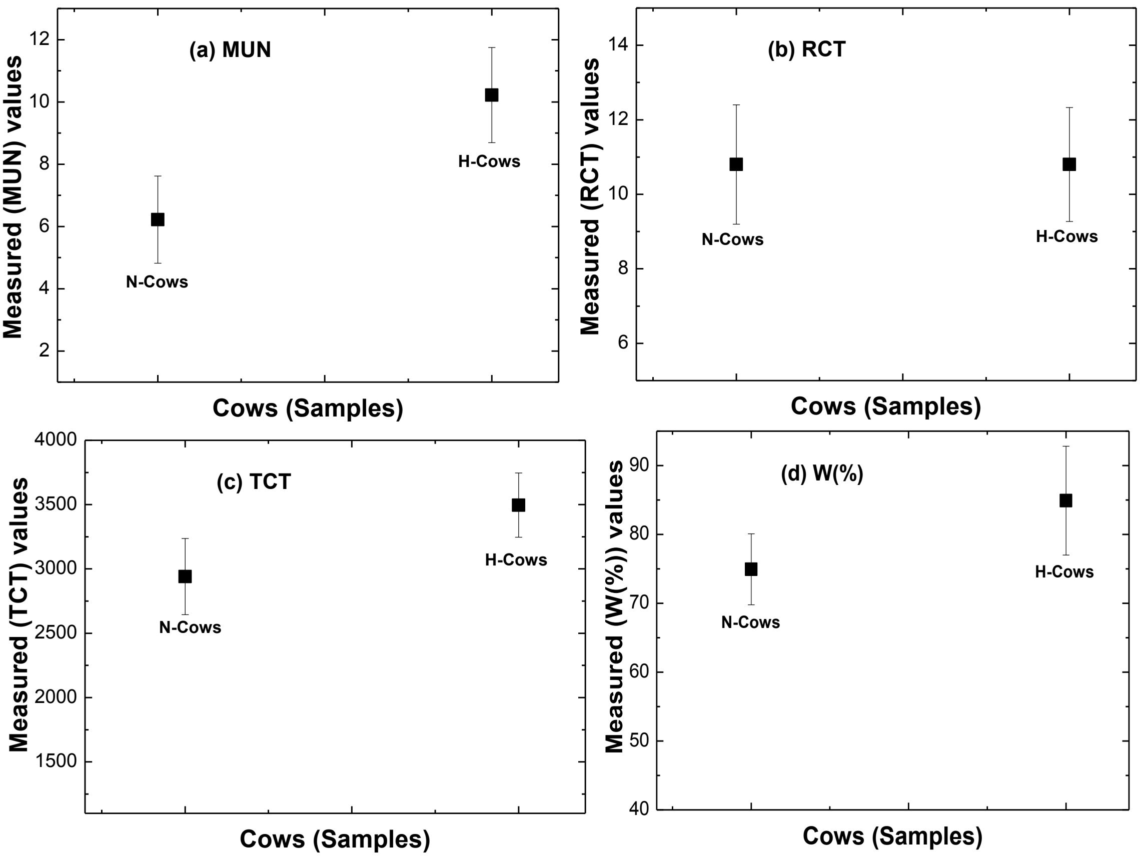


Figure 6 (a-d): For the statistically measured values of MUN, RCT, TCT and W (%) for both groups of cows.

Conclusions and final Remark

Based on our present statistical study, we can draw the following conclusions:

This study estimates for milk coagulation time and curd quality in this regard, we were of the same magnitude as those reported by Schaar (1984) [31], Caroli et al. (1990) [21] and Ikonen et al. (1997). This trial shows that nitrogen supply in the diet can influence the cheese making process. Milk urea is directly involved in milk clotting time, acidification kinetics and differences in curd quality. It has been realized significant differences between the groups of N-cows and H-cows, for various parameters, DMY, MUN, RCT, TCT and the quantity of separated whey. However, the technological quality was slightly better at cows with lower DMY. We can say that the high DMY reached by the genetic improvement of animals and their more efficient nutrition does not result in the noticeably impaired quality of raw milk. The results suggest that it would be interesting for the cheese makers to determine the urea content of milk in some particular periods of the year in order to choose their cheese making parameters.

Acknowledgment

The authors are very much gratefully acknowledge to farm workers and also farm authority to use the valuable facilities from the dairy farm, in Bitola region, R. Macedonia. And also the corresponding author Vesna Karapetkovska Hristova is highly thankful to the faculty members at the Trakia University, Stara Zagora, Bulgaria for providing her an opportunity to defense her Ph. D. thesis.

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