Correlation between phenotypic characteristics of chemical composition and rennet coagulation of sheep milk

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Abstract. The aim of the present study was to establish a correlation between phenotypic characteristics of chemical composition, somatic cell count and rennet coagulation of sheep milk during different stages of lactation. The experimental milk samples were taken from Bulgarian autochthonous sheep breed - Local Stara Zagora sheep, newly developed breed - Bulgarian dairy synthetic population (BDSP), and Lacaune sheep breed. From the data obtained about the chemical composition of sheep milk, it was established that Bulgarian dairy synthetic population sheep breed had the highest fat value – 9.50%, whereas for the other breeds the fat value was approximately 8.30%. A slight variation in values for protein, lactose, solids-non-fat (SNF) and density was observed in the individual milk samples. The determined somatic cell count in the experimental samples ranged from 11 400 to 9 560 866 cells/ml, as the average value for the whole lactation period was 643 259 cells/ml. Strong negative correlation between somatic cell count value and parameters of milk coagulation ability was also established – SSC:RCT (−0.170); SCC:K20 (−0.142) and SCC:A30 (−0.254). The collected data showed undeniably that in order to improve rennet coagulation of sheep milk, conduction of thorough research, analysis and evaluation of milk productivity was needed, as well as application of selective breeding approaches and reduction of somatic cell count in milk.

Key words: somatic cell count; phenotypic characteristics; chemical composition; rennet coagulation; sheep milk

1. Introduction

Selection and breeding of small ruminants is main sector in Bulgaria. Around 70–75% of the sheep gene pool in the country is represented by Bulgarian Dairy Synthetic Population breed. This large massif of animals is suitable for intensive industrial use, with high milk productivity potential. Therefore in last decade, there has been a growing interest in the production of traditional dairy products. Qualitative composition of milk from Bulgarian local sheep breeds, determines some good technical qualities for the production of this kind of identical dairy products. There are many studies aimed to revealing the genetic and phenotypic variation that characterize milk productivity in sheep’s [1, 2, 3, 4, 5, 6]. Variability of chemical composition and coagulation ability of sheep milk is determined by the main genetic factor – breed and a lot of non-genetic ones: feeding, season, technology of rearing the animals. To evaluate the potential of the animals, and to predict the future ups or downs of the productivity of the population, it have to take in count all the factors affecting the main productive traits [7].
When a mammary gland is affected i.e. there is an inflammatory process named mastitis [8, 9]. The causes of the disease are different kind of pathogenic microorganisms, which are found everywhere - in the soil, in the barns, in the floor and litter of sheepfolds, on the contaminated wool and skin of the udder, on the vessels and hands of milkers. So the hygienic control of milking (i.e., total bacterial count and somatic cell count) is related to the quality management control and monitoring in dairy farms.

The somatic cell count is one of the most important indicators of the quality of milk. It is also an index of udder health due to the high correlation with the degree of infection of the mammary gland - subclinical, chronic or acute mastitis.

The issue of human health at consumption milk with high levels of somatic cells is serious. These include antibiotic residues in milk, transfer antibiotic resistance from animal to human, and transfer of pathogens through milk or dairy products. Approximately 80% of the antibiotic residues in milk can be traced back to the treatment of mastitis [10].

The flow of milk constituents from blood to the lumen changes because of the different osmotic pressure (increase of lactose and mineral substances and decrease of fat and protein content in milk) and because of the increased total somatic cell count [11], hence the milk renneting properties are modified too [12]. Coagulation process in sheep’s milk is well when a number of factors are in normal range such as chemical composition, pH, titrable acidity, protein-micellar system, calcium concentration etc. [13].

Rennet coagulation of milk is a very important process in cheese manufacturing. The amount of milk used to manufacture cheese is growing worldwide, therefore a mechanism of rennet coagulation need to receive much attention in the dairy science and industry. Main process in cheese manufacturing is milk coagulation. Several works have confirmed the importance of rennet coagulation in terms of cheese processing, yield, and quality of the end products [14, 15]. There are several techniques described in the science which can be used to study rennet coagulation [16]. The rennet coagulation process consists of three main stages which parameters are usually determined by (1) rennet coagulation time (RCT, min), (2) time to curd firmness (CF) of 20 mm (k20, min), and (3) curd firmness 30 min after rennet addition (a30, mm) [17].

The aim of the present study was to establish correlation between phenotypic characteristics of chemical composition, SCC and rennet coagulation of sheep milk during different stages of lactation.

2. Materials and methods
The study was conducted in Agricultural Institute in Stara Zagora at Department: Breeding and technologies in sheep breeding. A total of 600 individual milk samples were examined, as milk was taken from 200 sheeps. Sampling was performed three times per month three consecutive months during lactation. The objects of the study were sheep farms in different regions of the country: Yambol, Stara Zagora, Haskovo and Plevn from Lacaune sheep breed, Local Stara Zagora sheep breed, and Bulgarian dairy synthetic population sheep breed. The experimental milk samples were taken from the morning milking, cooled and transported to a research laboratory in Agricultural Institute of Stara Zagora. Immediately before analysis, the samples were tempered and examined for main chemical and microbiological characteristics; their coagulation properties were also monitored.

The chemical composition of the milk (fat, solids-non-fat, protein, lactose and density) was determined by Ultrasound Lactoscan SFP Options Milk Analyzer.

In raw sheep milk the microbiological characteristics of somatic cell count were determined by Lactoscan – SCC Somatic Cell Counter - based on fluorescence microscopic counting of somatic cells. The diagram which describes mechanisms of rennet coagulation is shown in Figure 1. The main parameters of coagulation properties are measurements by computer program.
3. Results and discussion

Table 1 shows the basic statistic parameters (min. and max. value), standard deviation (SD) and coefficient of variation (CV) in chemical composition, value of SCC and rennet coagulation of sheep milk of Stara Zagora, Lacaune and Bulgarian dairy synthetic population sheep breeds. Over the study period it was established that the local Stara Zagora sheep had a lowest average level of protein 4.14% and SNF% 11.26% content but better coagulation properties were attributed to this breed. This breed stands out from the others with fastest rennet coagulation and curd firmness of 20 mm time, also hardest curd firm on 30 min. - RCT (8.56 min.); k20 (0.44 min.) and a30 (39.19 mm.) respectively. In contrast the French breed Lacaune indicated highest levels of protein and SNF content – 4.55% and 12.39% but a longer rennet coagulation time 12.36 min. The newly formed Bulgarian dairy synthetic population sheep breed had the highest average of fat content in milk 9.50% and rennet coagulation properties similar to Lacaune sheep breed. The obtained results corresponded to those established by other authors [19, 20], who studied the dynamics changes in the composition of sheep milk. From the data obtained it was evident that the density and lactose content were with lowest levels of coefficient of variation and standard deviation. Obtained results for the microbial parameter "Somatic Cell Count" (SCC) were used as an indicator of udder health, milk quality and main factor affecting on rennet coagulation properties of sheep’s milk. From the results it was evident that the Lacaune sheep breed had a highest level of SCC - 854 700 cells which correlated with a longer time for coagulation of milk. Stara Zagora sheep breed had a lowest average value of SCC - 571 357 cells and the fastest rennet coagulation time respectively. Our results were in agreement with several studies which reported that a higher level of SCC had a negative effect on milk composition properties and deterioration of rennet coagulation properties [21, 22, 23, 24, 25].

From the data obtained about the average chemical composition of sheep milk from different breeds (Figure 2), it was established that the average fat value was 8.30%, whereas the highest fat value showed Bulgarian dairy synthetic population sheep breed – 9.50%. A slight variation of the other characteristics was also established (protein, lactose, solids-non-fat, density) within narrow limits as the average values were: 4.28%, 6.37%, 11.63% and 36.46 for all sheep breeds. Characteristics of somatic cell count in sheep milk were very variable during the studied lactation period in all experimental milk samples. The established values between the individual samples ranged from 11 400 to 9 560 866 cells/ml. The average value of somatic cell count for the whole lactation period of the studied breeds of sheep milk was 643 259 cells/ml. Rennet coagulation time (RCT) of sheep milk in all experimental samples was established. It varied on the average between 9.5 and 10.08 min.; time to curd firmness (CF) of 20 mm (K20) – 0.48 min and curd firmness 30 min after rennet addition (A30) – 37.99. Obtained results corresponded to those reported by [26, 27, 28] who concluded that the rennet coagulation properties of milk and the reduction of incidents from uncoagulated milk was a right selection of animals producing milk with a lower somatic cell content.
### Table 1. Statistical parameters on chemical composition, value of SCC and rennet coagulation milk from different sheep breeds.

| Properties      | Type of sheep breeds |           |           |           |           |           |
|-----------------|----------------------|-----------|-----------|-----------|-----------|-----------|
|                 |                      | Stara Zagora sheep breed | Lacaune sheep breed | Bulgarian dairy synthetic population breed |
| Fat, %          | Average              | 7.725     | 6.990     | 9.50      |           |           |
|                 | Min.                 | 3.71      | 4.07      | 6.01      |           |           |
|                 | Max.                 | 12.25     | 9.15      | 15.78     |           |           |
|                 | SD                   | 1.790     | 1.379     | 1.697     |           |           |
|                 | CV                   | 3.205     | 1.967     | 2.883     |           |           |
|                 |                      | 11.26     | 12.390    | 11.60     |           |           |
| SNF, %          | Average              | 9.69      | 10.54     | 8.10      |           |           |
|                 | Min.                 | 3.58      | 5.28      | 3.86      |           |           |
|                 | Max.                 | 13.22     | 7.23      | 5.17      |           |           |
|                 | SD                   | 0.838     | 0.869     | 0.777     |           |           |
|                 | CV                   | 0.703     | 0.107     | 0.604     |           |           |
|                 | Density, °A          | 35.60     | 34.471    | 35.26     |           |           |
|                 |                      | 27.82     | 35.27     | 24.70     |           |           |
|                 |                      | 42.20     | 47.39     | 40.44     |           |           |
|                 |                      | 2.873     | 2.904     | 2.961     |           |           |
|                 |                      | 8.258     | 0.357     | 8.770     |           |           |
| Protein, %      | Average              | 4.14      | 6.55      | 4.28      |           |           |
|                 | Min.                 | 3.58      | 3.86      | 2.38      |           |           |
|                 | Max.                 | 4.88      | 5.17      | 4.80      |           |           |
|                 | SD                   | 0.312     | 0.320     | 0.383     |           |           |
|                 | CV                   | 0.095     | 0.039     | 0.147     |           |           |
| Lactose, %      | Average              | 6.16      | 6.79      | 6.35      |           |           |
|                 | Min.                 | 5.28      | 5.79      | 4.42      |           |           |
|                 | Max.                 | 7.23      | 7.17      | 7.62      |           |           |
|                 | SD                   | 0.459     | 0.476     | 0.438     |           |           |
|                 | CV                   | 0.210     | 0.058     | 0.192     |           |           |
| SCC, cell/ml    | Average              | 571 357   | 854 700   | 604 647   |           |           |
|                 | Min.                 | 19 114    | 11 403    | 20 852    |           |           |
|                 | Max.                 | 9 560 866 | 8 941 290 | 3 800 940 |           |           |
|                 | SD                   | 1 424 393 | 1 942 280 | 966 167   |           |           |
|                 | CV                   | -         | 239 180   | -         |           |           |
| RCTmin          |                      | 8.56      | 12.36     | 38        |           |           |
| k20min          |                      | 0         | 0.51      | 0         |           |           |
| a30mm           |                      | 0.44      | 0.51      | 38        |           |           |
|                 |                      | 0         | 0         | 0         |           |           |
|                 |                      | 72        | 80        | 22.420    |           |           |
|                 |                      | 17.034    | 22.420    | 22.420    |           |           |
|                 |                      | 2.158     | 2.760     | 2.760     |           |           |
|                 |                      | 4.562     | 7.346     | 22.420    |           |           |
|                 |                      | 20.817    | 0.904     | 2.760     |           |           |
|                 |                      | 1           | 0.074     |           |           |           |

*SD standard deviation; *CV coefficient of variation
Figure 2. Average value of chemical composition and rennet coagulation properties from all type sheep breeds.

The Table 2 presents the data for phenotypic correlations between chemical composition, SCC and enzymatic coagulation of sheep milk. The conducted phenotypic correlations analysis determined moderately negative to strong positive values of the reported values of the characteristics.

Table 2. Phenotypic correlations between chemical composition and enzymatic coagulation of sheep milk.

| Fat, % | SNF, % | Density, ºA | Protein, % | Lactose, % | SCC, cells/ml | RCT, min | K20, min | A30, mm | Fat, % | SNF, % | Density, ºA | Protein, % | Lactose, % | SCC, cells/ml | RCT, min | K20, min | A30, mm |
|-------|--------|-------------|------------|------------|---------------|----------|----------|--------|-------|--------|-------------|------------|------------|---------------|----------|----------|--------|
| 0.171 | -0.305 | 0.157       | 0.127      | -0.078     | 0.221         | 0.040    | 0.023    |        |       |       |             |            |            |                |          |          |        |
| 0.869 | 0.856  | 0.956       | -0.175     | 0.265      | 0.116         | 0.074    |          |        |       |       |             |            |            |                |          |          |        |
| 0.748 | 0.880  | -0.139      | 0.117      | 0.064      | 0.043         |          |          |        |       |       |             |            |            |                |          |          |        |
| 0.849 | -0.158 | 0.218       | 0.131      | -0.009     | -0.099        |          |          |        |       |       |             |            |            |                |          |          |        |
| -0.185| 0.220  | 0.105       | 0.029      |          |              |          |          |        |       |       |             |            |            |                |          |          |        |
| -0.170| -0.142 | -0.254      |          |            |              |          |          |        |       |       |             |            |            |                |          |          |        |
| 0.445 | 0.388  | -0.122      |          |            |              |          |          |        |       |       |             |            |            |                |          |          |        |

The data indicated that the main correlations between the chemical characteristics: SNF:protein, SNF:lactose, and SNF:density had strong positive correlations – 0.856; 0.956; 0.869. Similar results were confirmed by another author's research, which found a positive correlation between the curd firmness and the protein content (r = 0.310) [17]. Positive correlations were also determined for protein:lactose - 0.849; protein:density – 0.748, as well as lactose:density – 0.880. Weak to moderate
negative correlations in somatic cell count were identified and reported with all chemical characteristics, included in the study, from −0.078 to −0.254. The reported correlations between SCC:RCT (−0.170); SCC:K20 (−0.142) and SCC:A30 (−0.254) were analytically observed.

4. Conclusions
1. The collected data showed undeniably that in order to improve rennet coagulation of sheep milk, conduction of thorough research, analysis and evaluation of milk productivity was needed, as well as application of selective breeding approaches and reduction of somatic cell count in milk.
2. There was a negative correlation between SCC:RCT (−0.170); SCC:K20 (−0.142) and SCC:A30 (−0.254).
3. According to the obtained results SCC was a factor affecting coagulation ability of sheep milk.
4. Analysis of the results showed that lower average levels of protein and SNF content had no negative effect on the indicators determining the coagulation ability of milk.

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