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Relationships between milk yield, quality and coagulation properties with kappa-casein (κ-CN) genotypes of Bulgarian Rhodope cattle breed

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Abstract. *The aim of the present study was to establish the association between different κ-CN genotypes with milk yield, quality and coagulation properties in dairy cows of Bulgarian Rhodope breed. For determination of milk proteins polymorphism and its relationship with individual coagulation properties of milk and its quality, 136 milk samples from Bulgarian Rhodope dairy cows reared in 4 farms were collected. The visit to the farms and the taking of the milk samples took place between May and June 2009. Animal milk is based on daily milking on the relevant control day of the current lactation. During the morning milking, 50mL samples were collected without preservative, stored in cooling bags and analyzed in the specialized lab at the Agricultural Institute, Stara Zagora by Computerized Renneting Metter. The evaluated parameters of milk coagulation properties were: rennet coagulation time (RCT, min); curd firming time (K20, min) and curd firmness (A30, mm). Milk proteins polymorphism was identified by tissue samples collection from 136 cows. To this end, specialized pliers and marks with a vial containing desiccant were used. With this technique, the tissue sample is obtained and sealed at the time of identification of the animal. After collection, tissue samples were shipped to the University in Padova, Italy for PCR-RFLP analysis. DNA was purified using a Maxwell®16 Tissue DNA purification kit (Promega) according to the manufacturer's instructions. Data were analyzed using descriptive statistical analysis (SYSTAT 13). The results obtained give reason to the following conclusions: The highest milk yield was established in cows from the AH genotype – 16kg, whereas the lowest value was detected in cows from the BH genotype – 12.83kg. Homozygous cows from the BB genotype produced milk with the highest fat content – 5.11%, whereas heterozygous from the BH genotype: milk with the lowest protein content – 3.16%. The cows from the AH genotypes were outlined with the lowest milk fat and protein content. The longest rennet coagulation time (RCT) was observed in AA and AH genotypes, the longest curd firming time (K20) – in genotype AH, the highest curd firmness (A30) – in the milk of cows from the AB and BB genotypes.*

Keywords: autochthonous breed, milk production, rennet coagulation time, curd firming time, curd firmness, milk proteins polymorphism

Introduction

Bulgarian Rhodope cattle breed was recognized in 1989. Nowadays, it is the only dairy cattle breed in the Rhodope Mountains, closely associated with the way of living of local inhabitants. The breed is a typical representative of small dairy cattle breeds. The Bulgarian Rhodope cattle is created on the basis of Rhodope Shorthorn cattle through a complex reproduction crossing scheme with the Sofia Brown cattle, Obirental, Ayrshire and other cattle breeds, with Danish Jersey being the main improver (Nikolov et al., 2012).

Casein is the most important milk protein with regard to cheese making. Casein is the main milk protein and exists in several molecular forms (Alipanah et al., 2005). Specific cow milk casein protein fractions comprise: α s1 casein (CSN1S1), α s2 casein (CSN1S2), β casein (CSN2) and κ- caseins (CSN3) as insoluble fractions, α lacto albumin (LALBA) and β lacto globulin (LGB), which are classified as soluble fractions (Galila and Darwish, 2008). The determination of CSN3 alleles A and B is of practical significance, as allele B is associated with milk yield and yield of cheese production (Sulimova et al., 2007; Zambrano et al., 2010).

Gurses et al. (2016) found out that κ-CN genotypes were associated with higher protein content and nonfat dry milk solids in the milk of Holstein and Swiss Brown cattle, while in Jersey cattle – with higher milk fat content.

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The genetic aspect of milk proteins polymorphisms and their frequency in Bulgarian Rhodope cattle populations were investigated by Hristov et al. (2008, 2012, 2013, 2014) and Yordanova et al. (2013). The relationships between κ-casein polymorphisms and milk coagulation properties were subject to numerous studies (Kubarsepp et al., 2005; Hallen et al., 2008; Bonfatti et al., 2010; Yordanova, 2015; Karabashev, 2016; Petrovska et al., 2017). So far, no studies have been carried out in Bulgaria concerning the genetic polymorphism of milk proteins and its relationship to milk coagulation properties in Bulgarian Rhodope cattle.

The aim of the present study was to establish the association between different κ-CN genotypes with milk yield, quality and coagulation properties in dairy cows of Bulgarian Rhodope breed.

Material and methods

For determination of milk proteins polymorphism and its relationship with individual coagulation properties of milk and its quality, 136 milk samples from Bulgarian Rhodope dairy cows reared in 4 farms were collected. The distribution of samples per farms was as follows: farm 1 – 32 samples, farm 2 – 10 samples, farm 3 – 77 samples and farm 4 – 17 samples. The visit to the farms and the taking of the milk samples took place between May and June 2009. Animal milk is based on daily milk on the

relevant control day of the current lactation. In Farms 1, 3 and 4 the cows are reared tied. Feeding is based on corn silage and hay, and milking is twice via a central milking line. After milking, the animals are allowed to walk in the pastures gathered for this purpose. In Farm 2 cows are reared freely – in cubicles with individual lying pens. Feeding is also based on corn silage and hay, and milking is twice via a central milking line.

During the morning milking, 50mL samples were collected without preservative, stored in cooling bags and analyzed in the specialized lab at the Agricultural Institute – Stara Zagora. From each of the samples, 10mL milk was taken, heated to 35°C, then 0.2mL chymusin (NATUREN Plus 215/0.8L) was added and analyzed on a Computerized Renneting Metter. The evaluated parameters of the milk coagulation properties were: rennet coagulation time (RCT, min); curd firming time (K_{20}, min) and curd firmness (A_{30}, mm) (Figure 1).

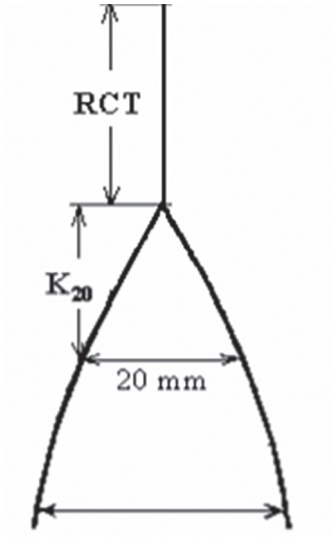


Figure 1. Parameters characterizing coagulation properties of milk (as per Kubarsepp et al., 2005).

Milk proteins polymorphism was identified by tissue samples collection from 136 cows. To this end, specialized pliers and marks with a vial containing desiccant were used. With this technique, the tissue sample is obtained and sealed at the time of identification of the animal. After collection, tissue samples were shipped to the University in Padova, Italy for PCR-RFLP analysis. DNA was purified using a Maxwell®16 Tissue DNA purification kit (Promega) according to the manufacturer's instructions. DNA is precipitated with three volumes of 70% cold ethanol, 0.1m sodium acetate (pH 5.2) and 2μL glycogen 2μg/mL, the pellets are then washed twice in 70% cold ethanol and dissolved in 50μL of dH2O. The DNA concentration is determined by a Qubit® fluorimeter (Invitrogen). A 221 bp fragment of exon IV was amplified using the following primers:

- Bub - F 5'-TGCCAAGCCCAGCCAACTACC-3'
- Bub-R 5'-CGACGGTTGAAGTAACTTGGGCTG-3'.

The screening was performed by a Genetic CEQ8000 assay system (Beckman Coulter), and the single nucleotide polymorphism (SNP) determination was performed with Beckman Coulter genetic analysis software v.9.00.

Data were analyzed using descriptive statistical analysis (SYSTAT 13).

Results and discussion

Percentage distribution of different genotype animals was presented on Figure 2. It is evident that animals with the genotype AB have the highest distribution rate - 56.36%. Genotypes AA and BB reported close results - 19.05% and 19.84%. Unlike us, Kučerova et al. (2006) establish the presence of 3 alleles in an experiment with Simmental cows, but they also found allele E. The genotypes of κ-CN are AA, AB, BB, AE and BE, the most common genotype being AB. The authors conclude the positive effect of allele B and genotype BB on κ-CN on the protein and fat content of milk.

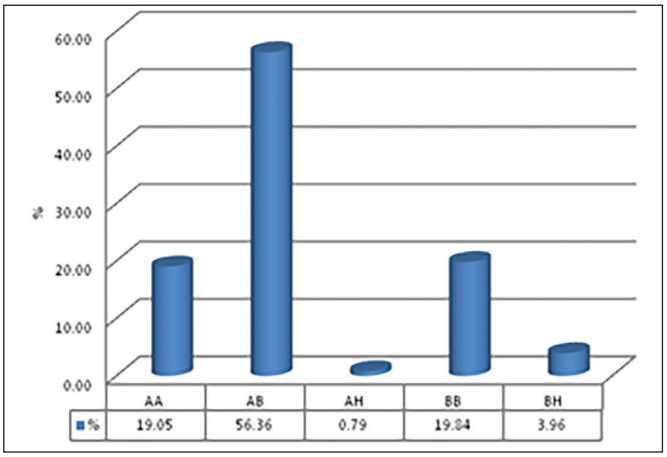


Figure 2. Percentage distribution of different genotype animals

The average test-day milk yield in Bulgarian Rhodope cows from the different κ-CN genotypes is presented on Figure 3. Heterozygous animals with AH genotype exhibited the highest milk yield – 16.00kg, whereas the lowest values were observed in cows with the BH genotype – 12.83kg. In two genotypes: AA and AB, the values of the studied trait were very close: AA – 14.19kg and AB – 14.66kg, while the average test-day milk yield in cows with BB genotype was 13.11kg. Hristov et al. (2014) reported comparable results in Bulgarian Rhodope cattle: highest milk yield in heterozygous cows from the AB genotype – 4099kg, followed by homozygous AA cows – 3896kg.

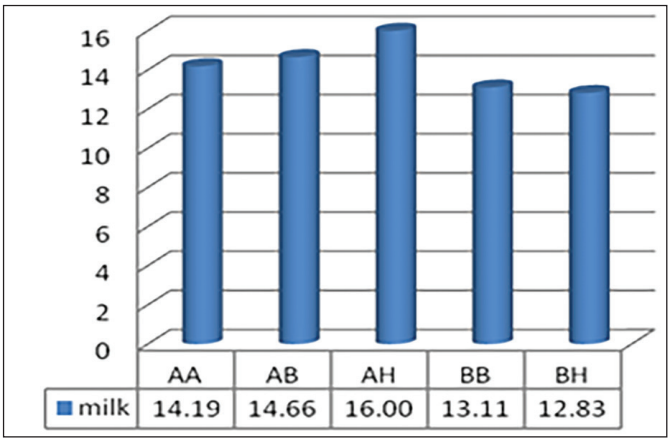
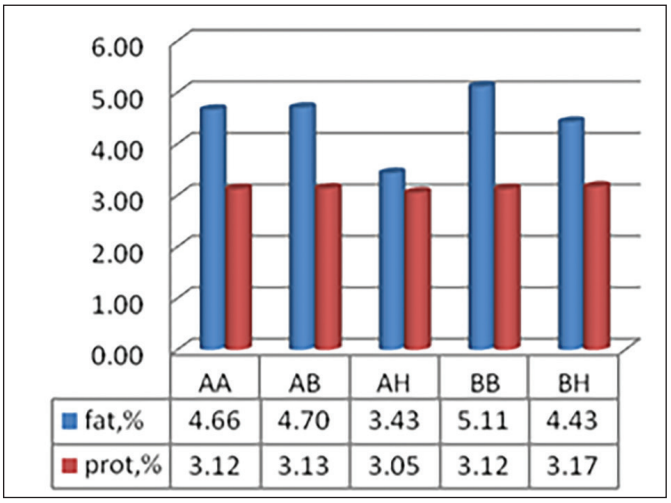


Figure 3. Milk yield in Bulgarian Rhodope cattle cows from different κ-CN genotype

Milk fat percentage (Figure 4) was the highest in heterozygous AB cows – 4.70%, followed by the homozygous genotype AA – 4.66%, and BH genotype – 4.43%. The lowest milk fat content as well as milk protein percentage were found in the AH genotype, 3.43% and 3.05%, respectively. The highest average milk protein content was demonstrated in cows from the NH genotype – 3.16%. The other genotypes showed lower values: AB – 3.13%, AA – 3.12% and BB – 3.12%. The milk produced by heterozygous animals from the AH genotype was characterized by the lowest milk protein content – 3.05%.

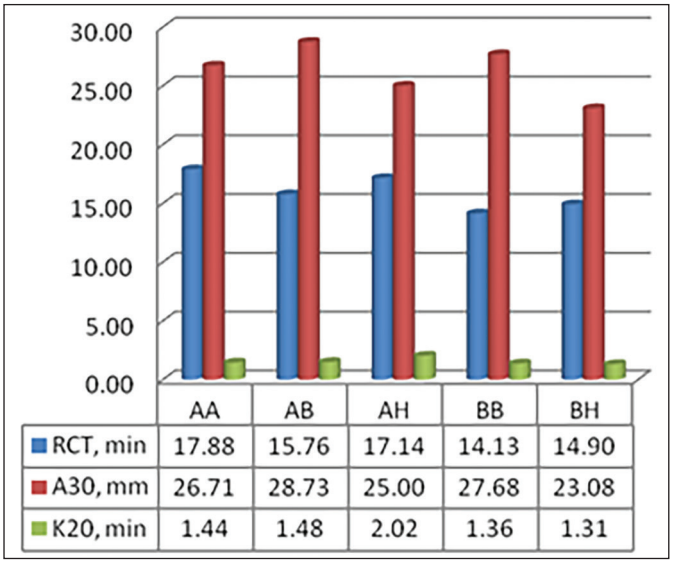


	AA	AB	AH	BB	BH
fat,%	4.66	4.70	3.43	5.11	4.43
prot,%	3.12	3.13	3.05	3.12	3.17

Figure 4. Milk quality in Bulgarian Rhodope cattle cows from different κ-CN genotypes

Hristov et al. (2014) reported different data affirming that the milk of heterozygous animals was with the lowest protein and fat content: – 3.54 and 4.58%, respectively, whereas the milk of genotype AA had the highest protein and fat content (3.76 and 4.78%). These differences were probably attributed to the fact that although animals with more Jersey blood predominated, due to the longer life span of cattle heterogeneous cows were also present. In the 80 animals studied by the authors, alleles other than A and B were not established, as well as resultant genotypes. Our results corresponded to the conclusions of Ilie et al. (2009), summarizing that the BB genotype of κ-CN was positively associated with milk protein content in Brown and Simmental cattle breeds.

Figure 5 shows that the cows from the BB genotype were characterized with the lowest rennet coagulation time – 14.13min, whereas the slowest coagulation of milk was observed in homozygous cows from the AA genotype – 17.88min. Intermediate rennet coagulation times were found out in heterozygous AB, AH and BH genotypes: 15.76, 17.14 and 14.9min, respectively.



	AA	AB	AH	BB	BH
RCT, min	17.88	15.76	17.14	14.13	14.90
A30, mm	26.71	28.73	25.00	27.68	23.08
K20, min	1.44	1.48	2.02	1.36	1.31

Figure 5. Milk coagulation properties on Bulgarian Rhodope cattle cows from different κ-CN genotypes

Curd firmness was the highest in cows from the AB (28.73mm), BB (27.68mm) and AA (26.71mm) genotypes, and with lower values in genotypes AH and BH – 23.08 and 25.00mm. The third parameter of milk coagulation properties: curd firmness time (K_{20}) ranged from 1.305 min (BH) to 2.02 min (AH). Intermediate values were found out in the other three genotypes: AA, AB and BB – 1.44, 1.48 and 1.36min, respectively. The highest curd firmness was that of the milk of cows from AB and BB genotypes. This is also confirmed by previously reported data showing that the milk of cows from BB genotype from Estonian dairy cattle breeds had significantly superior milk coagulation parameters from technological point of view (Joudi et al., 2009). Celik (2003) and Hallen et al. (2008) demonstrated that the different variants of κ–casein had positive effect on milk protein content, casein content, cheese yield and curd firmness.

Conclusion

The results obtained give reason to the following conclusions: The highest milk yield was established in cows from the AH genotype – 16kg, whereas the lowest value was detected in cows from the BH genotype – 12.83kg. Homozygous cows from the BB genotype produced milk with the highest fat content – 5.11%, whereas heterozygous ones from the BH genotype: milk with the lowest protein content – 3.16%. The cows from the AH genotypes were outlined with the lowest milk fat and protein content. The longest rennet coagulation time (RCT) was observed in AA and AH genotypes, the longest curd firming time (K_{20}) – in genotype AH, the highest curd firmness (A_{30}) – in the milk of cows from the AB and BB genotypes.

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