

Long-term changes in the frequency of beta-casein, kappa-casein and beta-lactoglobulin alleles in Polish Holstein-Friesian dairy cattle

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ABSTRACT. The purpose of the study was to analyse changes in the frequency of genotypes and alleles of beta-casein (*CASB*), kappa-casein (*CSN3*) and beta-lactoglobulin (*LGB*) loci among bulls (used for insemination in 1991–2021) and cows (milked in 2014 and 2021). The analysis included 1812 bulls and 2628 cows of the Polish Holstein-Friesian breed. Genotypes of three milk protein loci were identified using PCR-RFLP/ACRS method (bulls) or the Illumina Bovine MDv2 Chip (cows). For bulls used in insemination between 1991 and 2021, the frequency of the desirable *CSN3* allele B and allele A2 at the *CASB* locus increased by 44% and 8%, respectively. In cows, the frequency of allele B of *CSN3* and allele A2 of *CASB* increased over the past seven years by 11% and 7%, respectively. The frequency of *LGB* allele B decreased in cows from 47% to 41%. An increase in the frequency of the rarest allele E at the *CSN3* locus in bulls was also observed (from 2% to 7%). The number of animals carrying the most desirable combination of *CASB* and *CSN3* genotypes (A2A2 and BB) reached 4.25% and 2.8% in cows and bulls, respectively. The current results showed a slow but marked increase in the desirable alleles at casein gene loci which should improve milk quality and cheese-processing properties. This positive effect was attenuated by the simultaneous decrease in the frequency of *LGB* allele B.

Introduction

Milk is a complex food, making an important contribution to the body's requirement for basic nutrients: carbohydrates, lipids, proteins, vitamins and minerals. Six fractions of milk proteins have been identified, which occur in many genetic variants (Roginsky, 2003). Milk protein variants in dairy cattle have been the subject of research for 67 years (Aschaffenburg and Drewry, 1955), and some of them have been found to affect milk properties. For example, of three variants (A, B, E) identified in the kappa-casein (*CSN3*) gene,

variant B has been widely proven to contribute to better milk processing traits (Jakob and Puhán, 1992; Heck et al., 2009). Similar effects have been shown for variant B in the beta-lactoglobulin (*LGB*) gene (Caroli et al., 2009). In contrast, digestion of variant A1 of beta-casein (*CASB*) in the small intestine is known to result in the release of beta-casomorphin-7 peptide, which is believed to increase the risk of certain human diseases (reviewed by Kamiński et al., 2007; Cieślińska et al., 2012; Sheng et al., 2019; Ramakrishnan et al., 2020).

The main objectives in dairy cattle breeding programmes have focused on production (e.g. milk

yield) or functional traits (e.g. fertility). Progress in developing these traits over many generations may have induced changes in the frequencies of allele not directly associated with the traits under selection, even though they may later be considered desirable for other reasons. Monitoring the fluctuations of such alleles over a long period of time seems necessary to avoid the loss of genetic diversity and milk attributes important for the dairy industry to maintain high quality of dairy products. To date, such analyses have been reported for French (Sanchez et al., 2020) and Italian (Sebastiani et al., 2020) dairy cattle.

The aim of the study was to analyse changes in the frequency of *CASB*, *CSN3* and *LGB* alleles in bulls and cows of Polish Holstein-Friesian cattle.

Material and methods

The analysis included 1824 bulls and 2628 cows of the Polish Holstein-Friesian breed (Black-and-White variety). The bulls were used for artificial insemination of cows in Poland in 1991–2021, with different time intervals depending on the milk protein gene. The *CSN3*, *CASB* and *LGB* genotypes of bulls were determined in 1991–2021, 2003–2019 and 1991–2014, respectively. The genotypes of the three milk protein genes in cows were determined twice: in 2014 and 2021 in cows kept in 31 herds in different parts of Poland.

Genomic DNA of bulls was isolated from commercial semen straw using the Wizard DNA Purification Kit (Promega, Madison, WI, USA). Genomic DNA of cows was isolated with the NucleoSpin Tissue Mini kit (Macherey-Nagel, Allentown, PA, USA) from ear tissue samples collected from cows using Allflex technology. Semen and ear tissue samples were collected during official routine veterinary examinations. The procedures used in the experiment were in accordance with ethical guidelines (Local Ethics Committee approval No. 50/2015/N). Genotypes of three milk protein loci were identified in bulls by self-developed PCR methods (Kamiński and Figiel, 1993; Kamiński and Zabołewicz, 1997; Cieślińska et al., 2012). Cow genotypes were identified using an Illumina Bovine MDv2 Chip (Illumina, San Diego, CA, USA). For the set of SNPs present on the microarray, a preliminary quality data analysis was performed using Illumina GenomeStudio (version 2011.1; Illumina, San Diego, CA, USA)

software. First, all SNPs whose clusters were not separated were removed. Subsequently, all SNPs with Call Frequency below 0.99 and clusters with too low mean normalised intensity signal to reliably call genotypes were zeroed. Additionally, the correctness of the genotypes was confirmed by checking the concordance between duplicated SNPs on the same chip. In all samples, SNPs at the *CSN3*, *CASB* and *LGB* loci were successfully genotyped. Frequency of genotypes (F_G) and frequency of alleles (F_A) were calculated using the following formulas:

$$F_G = n / N,$$

where: n – the number of individuals with certain genotype and N – the number of all individuals;

$$F_A = f_x / 2N,$$

where: f_x – the number of alleles x and $2N$ – total number of all alleles in the population.

The Hardy-Weinberg (HW) equilibrium was verified using the Chi-square test ($P < 0.05$).

Results

Thirty years of genotyping bulls for *CSN3* polymorphism has shown a distinct trend in allele frequencies. Three alleles were identified in 1824 bulls: A, B and E. The frequency of the desirable allele B of the *CSN3* gene increased from 19% (1991) to 63% (2021) (Figure 1). For *CASB*, two alleles were identified among 877 bulls, i.e. A1 and A2. Although the *CASB* polymorphism was investigated for a shorter period, an increase in the frequency of the desirable allele A2 was recorded (from 61% in 2003 to 69% in 2019) (Figure 2). The *LGB* polymorphism was identified in 939 bulls between 1991 and 2014, and showed a decrease in the frequency of the desirable allele B (from 63% to 40%) (Figure 3).

Figure 4 depicts changes in the frequencies of *CSN3*, *CSN2* and *LGB* alleles in the population of 2628 cows. For *CSN3*, the frequency of the desirable allele B increased in the last seven years from 31% in 2014 to 42% in 2021. An increase in the frequency of allele E from 8% to 11% was also recorded. For *CASB*, the frequency of the undesirable allele A1 decreased from 49% (in 2014) to 33% (in 2021). We were not able to determine changes in the frequency of allele I of the *CASB* gene in cows, as it was only genotyped in 2021 and its frequency was 8%. The frequency of *LGB* allele B decreased in cows from 47% to 41%.

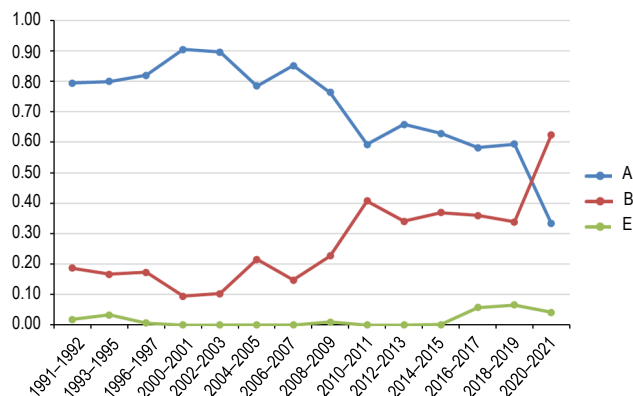


Figure 1. Fluctuations of kappa-casein allele frequencies in Polish Holstein-Friesian bulls used in insemination between 1991 and 2021
A – allele A, B – allele B, E – allele E

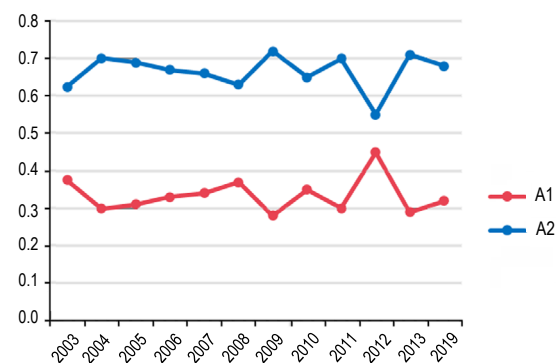


Figure 2. Fluctuations of beta-casein allele frequencies in Polish Holstein-Friesian bulls used in insemination between 2003 and 2019
A1 – allele A1, A2 – allele A2

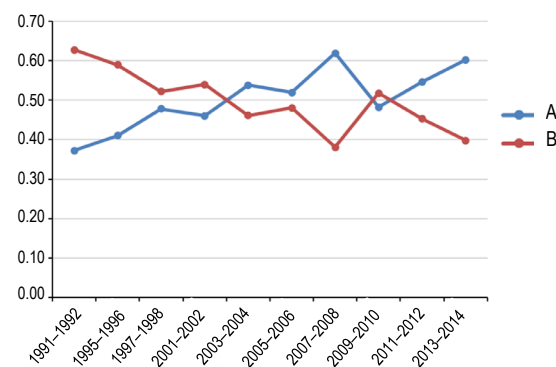


Figure 3. Fluctuations of beta-lactoglobulin allele frequencies in Polish Holstein-Friesian bulls used in insemination between 1991 and 2014
A – allele A, B – allele B

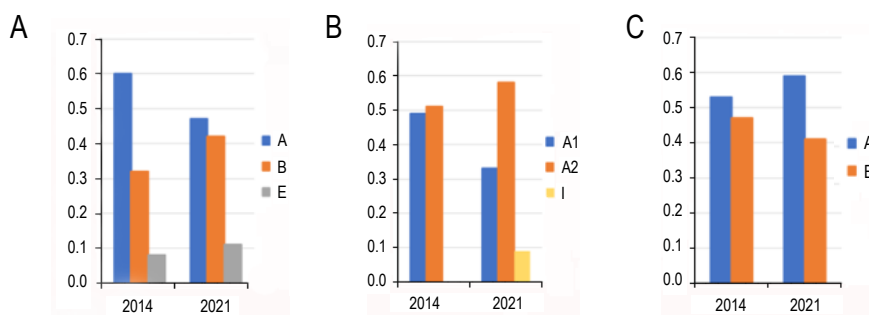


Figure 4. Frequencies of kappa-casein (A), beta-casein (B) and beta-lactoglobulin (C) alleles in Polish Holstein-Friesian cows
A1 – allele A1, A2 – allele A2, I – allele

The most desirable combination of *CASB*-*CSN3* genotypes (A2A2-BB) was found in 2.8% of bulls and 4.25% of cows (data not shown).

Discussion

It is commonly believed that genetic polymorphisms of milk proteins in dairy cattle affect important milk properties and play a key role in cheese-making (Roginski, 2003). Therefore, artificial insemination companies began genotyping dairy bulls for kappa-casein in the early 1990s (Denicourt et al., 1990; Kamiński and Figiel, 1993). Information on the *CSN3* genotype has been made widely available in commercial catalogues of bulls, and those with the desirable BB genotype were promoted as “cheese-making bulls”. Later, the *LGB* gene polymorphism was added to *CSN3* as another marker of improved milk processing characteristics. Finally, in the last ten years, the *CASB* polymorphism has also started to be determined in bulls and cows due to the undesirable effects of allele A1 on human health (McLachlan, 2001). Breeding centres in Poland joined these efforts at different times to find and promote bulls carrying the most advantageous genotypes (BB, A2A2 and BB) for *CSN3*, *CASB* and *LGB*, respectively. The authors participated in these activities in the years 1991–2021, and the present study shows trends in allele frequency changes in Polish Holstein-Friesian bulls and cows. In general, the frequency of the most desirable *CSN3* (B) and *CASB* (A2) alleles are steadily increasing in bulls and cows. This positive effect on milk quality is slightly weakened by a decrease in the frequency of *LGB* allele B, which declined by 23% and 6% in bulls and in cows, respectively. Similar analyses were conducted for different breeds of French dairy cattle (Sanchez et al., 2020). In French Holstein bulls, the frequency of *CSN3* allele B increased from 11% (in 1990) to 39% (in 2020), thus this change over almost the same time interval was much slower in French Holstein than in Polish Holstein bulls. At the *CASB* locus, the frequency of allele A1 in French Holstein bulls decreased from 38% to 29% which was very similar to the value observed in Polish Holstein bulls, although the time interval in the current study was 13 years shorter. Finally, the frequency of *LGB* allele B decreased in French Holstein bulls from 59% to 33%, which was similar to the trend observed in the current study (63% to 40%), but the monitoring was discontinued much earlier (in 2014). The datasets for cows vary considerably, since the genotypes of French Holstein and Polish Holstein-Friesian cows were collected continuously

from 2000 to 2015 and discontinuously in 2014 and 2021, respectively. Nevertheless, the frequency of *CSN3* allele B in French Holstein cows was at a stable level, reaching 41% in 2000 and 40% in 2015. For seven years, the frequency of the desirable allele B of *CSN3* in Polish Holstein-Friesian cows has increased by 11%. In *CASB*, the frequency of the undesirable allele A1 in bulls was also at a stable level of approx. 30% over 15 years, and its frequency decreased from 49% to 33% in Polish Holstein-Friesian cows. Lastly, the frequencies of *LGB* polymorphism in French and Polish Holstein cows differed slightly; for French Holstein cows, a decrease in allele B frequency from 43% to 40% was observed, while this decline was slightly higher in Polish Holstein cows (from 47% to 41%).

Kolenda and Sitkowska (2021) recently published a study on genotype and allele frequencies in casein genes based on 1900 Polish White- and Black Holstein cows milked in three herds between 2014 and 2016. Their results differed slightly compared to the current findings, e.g. the frequency of beta-casein allele A1 was estimated at 38%, which was lower than the value determined in the present study (49%). The difference could be due to the number of herds (3 vs 31). Moreover, the desirable allele B of the *CSN3* polymorphism showed almost the same frequency, i.e. 40%, which was similar to the current study (42% in 2021). Allele A1 in Italian Holstein cattle (Sabastiani et al., 2020) had a frequency of 30%, which again resembled the present results (33% in 2021).

The observed changes in allele frequencies could be the effect of breeders' decisions and/or a positive correlation between certain milk protein variants and milk production traits. The latter option was confirmed in the authors' previous reports which showed that *CASB* allele A1 was associated with lower milk yield, while variant A2 increased breeding values for milk and protein yield – traits with the highest weight in the Polish selection index (Oleński et al., 2010; 2012). Moreover, Nilsen et al. (2009) suggested that the increasing frequency of *CASB* allele A2 in the Norwegian cattle population was due to its positive effect on milk yield. Similar results were also reported by Heck et al. (2009) in Dutch dairy cattle. Additional benefits of variant A2 were also economically estimated by Morris et al. (2005), who indicated that A2A2 milk had a higher daily yield (by about 2.1%) than milk from cows carrying the A1A1 or A1A2 genotype. Furthermore, Kearney et al. (2005) calculated that A2A2 cows were more profitable in terms of milk yield

compared to A1A2 or A1A1 cows. These reports demonstrated that preferring *CASB* variant A2 in selection and reproduction would not negatively affect milk production. In other words, milk from cows A2A2 would be profitable and additionally “healthier” than milk containing variants A1 and A2. To achieve this goal, systematic mating of all cows in the herd with A2A2 bulls should be conducted until a sufficient number of cows have the A2A2 genotype to begin separating milking and other logistical and organizational preparations for processing this type of milk in a dairy plant. This positive effect was weakened by the simultaneous decrease of allele B frequency at the *LGB* locus. Since the *LGB* gene is located on a different chromosome than the casein genes (Caroli et al., 2009), this change cannot be the result of linkage disequilibrium. One can only speculate that the proportion between casein and whey proteins (and their variants) must somehow be balanced in milk to preserve its basic properties and content.

Conclusions

The study demonstrated a slow but distinct increase in the frequency of the desirable *CSN3* and *CASB* alleles in Polish Holstein-Friesian cattle, which should improve the processing properties of milk, as well as its health-promoting value, thereby enabling the development of novel dairy products.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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