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ALLELE AND GENOTYPE FREQUENCIES OF MILKPROTEIN KAPPA-CASEIN (CSN3) IN ARTIFICAL INSEMINATION BULLS OF CZECH FLECKVIEH AND HOLSTEIN BREED

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Abstract

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This thesis was meant to evaluate allele and genotype frequencies of kappa-casein (CSN3) in artificial insemination bulls in the Czech Republic of Czech Fleckvieh breed (n = 133) and Holstein breed (n = 58). Studied bulls were born between 1990 and 2005. Calculation was made by PowerMarker (Liu K., Muse S.V. 2005). Genotype frequencies established in Czech Fleckvieh bulls were AA = 0.391 AB = 0.4887 AE = 0.0075 BB = 0.0902 BE = 0.0226 and EE = 0 and allele frequencies were A = 0.6391 B = 0.3459 E = 0.015. Genotype frequencies established in Holstein bulls were AA = 0.5517 AB = 0.2759 AE = 0.1207 BB = 0 BE = 0.0345 and EE = 0.0172 and allele frequencies A = 0.75 B = 0.1552 and E = 0.0948. In Holstein breed we found higher frequency of E allele and higher frequency of genotypes with this allele. Genetic diversity, heterozygosis and PIC values in Czech Fleckvieh Bulls were following 0.4717; 0.5188 and 0.3737 and 0.4044; 0.4310 and 0.3668 in Holstein Bulls. These results show the higher genetic variability in bulls of Czech Fleckvieh breed. Results of χ^2 test (Czech Fleckvieh 4.58; Holstein 2.55) show genetical disequilibrium in the Bulls both examined breeds used in artificial insemination in the Czech Republic.

kappa-casein, allele frequencies, genotype frequencies, Czech Fleckvieh bulls, Holstein bulls

Concerning bulls of Czech Fleckvieh and Holstein breed, which are active in an artificial insemination, determination of milkprotein kappa-casein (CSN3) is very important information. And it is not only for cow-breeders but also for farmers. The determination of the genotype is relevant in relation to milk, protein and fat yield and also for milk quality.

Kučerová et al. (2006) detected increased protein and fat content in milk in population of Czech Fleckvieh breed of BB genotype. In the observed population frequencies of CSN3 allele B (0.38) and BB genotype (13%) were detected. The effect of AA CSN3 genotype on milk, protein and fat yield and effect of AA genotype on protein and fat percentage was found by the author also in her former study (Kučerová et al., 2004). A significantly higher difference in protein production ($p < 0.05$) was determined between AB and BB CSN3 genotypes in the first lactation of dairy cows of the Slovak Fleckvieh breed (Žitný et al., 1996). Dairy cows with AB genotype produced on average by 17 kg of proteins more than the group of cows with BB CSN3 genotype. Eenennaam et al. (1991) obtained the highest milk yields for the BB genotype from 1454 first lactation heifers of California dairy cattle population. Negative effects of E allele on milk production parameters and pro-

tein quality were found by Ikonen et al., (2004). BB CSN3 genotype is also connected with higher cheese yield and better coagulation qualities (Hanuš, Beber; 1995).

Kučerová et al. (2006) established frequencies for CSN3 locus alleles A, B and E in Czech Fleckvieh cattle as 0.598 0.378 and 0.024. Frequencies of AA, AB, AE, BB and BE CSN3 genotypes were 35.4 46.8 2.3 13.0 2.5. The EE genotype was not found in Czech Fleckvieh cows. Bulls of the Czech Fleckvieh breed (n = 37) showed three CSN3 genotypes AA, AB and BB 38% 48% and 14% with frequencies of alleles A = 0.61 and B = 0.39 (Kučerová et al., 2004). Čítek et al. (1996) detected frequencies of the A and B alleles of CSN3 in 25 individuals of Bohemian Red strain. This old strain belongs to endangered gene reserves. Relative frequency of A allele was 0.52 and B allele 0.48. Relative frequency of the AA genotype was 0.24; AB 0.56 and BB 0.2. In the population of the Slovak Fleckvieh cows (Žitný et al., 1996) three genotypes were found – AA (28%), AB (59.7%) a BB (12.3%). In this study 717 cows were observed. Higher occurrence of AA genotypes (57%) was found in Polish Black-and-White cattle (Strzalkowska et al., 2002). Lower occurrence of genotypes AB (39%) and markedly low proportion of the BB genotypes (4%) were determined in this population. Genotypes of kappa-casein were analysed in three breeds in Austria. Frequencies of alleles were A = 0.73 and B = 0.27 in Fleckvieh, 0.36 and 0.64 in Braunvieh and 0.54 and 0.46 in Grauvieh. Frequencies of AA, AB and BB genotypes in analysed breeds were following: Fleckvieh 51.9% 42.8% 5.3% Braunvieh 14.2% 42.9% 42.9% and Grauvieh 26.1% 56.5% 17.4% (Schellander et al., 1992). In Canadian artifical insemination bulls (Holstein, Ayshire and Jersey) Sabour et al. (1993) reported frequencies of alleles A and B. Frequencies of B allele in these breeds were 0.14 0.22 and 0.92. AA, AB and BB genotype frequencies were 0.75 0.23 and 0.02 in Holstein breed, 0.58 0.39 and 0.03 in Ayshire breed and 0 0.15 and 0.85 in Jersey breed.

Čítek et al. (1996) compared relative and theoretical CSN3 genotype frequencies in Bohemian Red strain. Calculated χ^2 test value was 1.48 (in 25 individuals of the breed) and table values are P = 0.01 and $\chi^2 = 9.21$. It shows a statistically insignificant difference. A rate of homozygosis was 0.501.

MATERIAL AND METHODS

Allele and genotype frequencies were evaluated in 133 bulls of Czech Fleckvieh strain and 58 bulls of Holstein strain. The bulls, born between 1990 and 2005, were chosen for artificial insemination and they were a part of a dairy cattle population in the Czech Republic. The genotype was defined from their semen of sires. PowerMarker (Liu K., Muse S. V.: Integrated analysis environment for genetics marker data, Bioinformatics 21 /9/: 2128 – 2129, 2005) programe was used for the calculation. The calculation was based on finding allele and genotype frequencies in Czech Fleckvieh and Holstein bulls (including Variance and SD). Genetical equilibrium was reached by counting χ^2 test and LRT value (Hardy-Weiberg disequilibrium). Calculation of heterozygosis and PIC values (polymorphism information content) of the bulls of examined strains was a part of the procedure.

Polymorphism information content:

$$PIC_l = 1 - \sum_{u=1}^{k} \bar{p}_{lu}^{\,2} - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\bar{p}_{lu}^{\,2}\ \bar{p}_{lv}^{\,2}$$

Heterozygosity: $H_l = 1 - \sum_{u=1}^{k} \bar{P}_l$

Gene diversity: $D = (1 - \sum_{u=1}^{k} \bar{p}_{lu}^{\,2})$.

RESULTS AND DISCUSSION

Table No. I shows established relative allele frequencies (A = 0.6391 B = 0.3459 E = 0.015) and genotype frequencies (n = 133) in Czech Fleckvieh bulls (AA = 0.391 AB = 0.4887 AE = 0.0075 BB = 0.0902 BE = 0.0226 and EE = 0). These results support values sateted by Kučerová et al. (2004), who examined 37 bulls and declares following genotype frequencies in this strain: AA = 0.38 AB = 0.48 a BB = 0.14 and allele frequencies A = 0.61 a B = 0.39. Both results show higher rate of AB genotype and a low frequency of E allele genotype and absence of EE genotype. High rate of AB genotype (59.7%) was also found in population of Slovak Fleckvieh strain (Žitný et al., 1996) together with a lower rate of AA genotypes (28%) and higher rate of BB genotypes (12.3%). Austrian population of breeding bulls showed on the contrary higher rate of BB genotype in Fleckvieh (5.3%), Braunvieh (42.6%) and Grauvieh (17.4%) strain. B allele is not more frequent in Austrian Fleckvieh population (Fleckvieh in Austria 0.27; Fleckvieh in the Czech Republic 0.3459), because AB genotype is more frequent in Czech bulls.

Table No. II shows relative allele frequencies (A = 0.75 B = 0.1552 and E = 0.0948) and genotype frequencies (n = 58) in bulls of Holstein breed (AA = 0.5517 AB = 0.2759 AE = 0.1207 BB = 0 BE = 0.0345 and EE = 0.0172). Higher frequency of AA genotype and genotypes with E allele is apparent in Czech Fleckvieh strain population. Sabour et al. (1993) also stated higher frequency of AA genotype (0.75) in

Canadian Holstein bulls‘ population, but population of Holstein bulls in the Czech Republic is less numerous. Other genotype frequencies in Canadian bulls are comparable to the Czech ones (AB = 0.23 and BB = 0.02). Higher rate of AA genotype (0.57) was found in Polish Black-and-White cattle (Strzalkowska et al., 2002). Also a very low rate of BB genotypes (0.04%) was found in this population. Stated frequencies of A and B alleles were, in case of Polish Black-and-White cattle, the same as in this research (Polish Black-and-White cattle A = 0.77 B = 0.23; Czech Republic A = 0.75 B = 0.1552).

Holstein and Czech Fleckvieh bulls were chosen for insemination according to milk efficiency indicators (kg of milk, milk components). The selection of bulls is not therefore random. It is in accordance with χ^2 test results that show genetical disequilibrium in both examined breeds (Table No III). Comparing of genetical diversity and heterozygosis (Table No III) shows partially higher variability in Czech Fleckvieh cows (0.4717; 0.5188) in relation to Holstein (0.4044; 0.4310). Slihgtly higher value of polymorphism information content (PIC) in the Czech Fleckvieh cattle (0.3737) than in Holstein cattle (0.3668) reflects that fact.

I: *Genotype and allele frequencies of CNS3 in Bulls of Czech Fleckvieh breed (born 1990–2006)*

Genotype	n	Freq.	Cov.	Allele	Freq.	Variance	SD
AA	52	0.3910	0.0008	A	0.6391	0.0008	0.0283
AB	65	0.4887	0.0007	B	0.3459	0.0007	0.0273
AE	1	0.0075	0.0001	E	0.0150	0.0000	0.0074
BB	12	0.0902	0.0007				
BE	3	0.0226	0				
EE	0	0	0				
Total	133	1.00					

II: *Genotype and allele frequencies of CNS3 in Bulls of Holstein breed (born 1990–2006)*

Genotype	n	Freq.	Cov.	Allele	Freq.	Variance	SD
AA	32	0.5517	0.0015	A	0.7500	0.0015	0.0390
AB	16	0.2759	0.0008	B	0.1552	0.0009	0.0304
AE	7	0.1207	0.0007	E	0.0948	0.0008	0.0285
BB	0	0	0				
BE	2	0.0345	0.0001				
EE	1	0.0172	0.0008				
Total	58	1.00					

III: *Testing of genetical equilibrium of CSN3 genotypes in Bulls of Czech Fleckvieh and Holstein breed*

	Czech Fleckvieh	Holstein
Total	133	58
χ^2 test	4.5811	2.5501
χ^2 d.f.	3	3
χ^2 p-value	0.2092	0.4663
Exact p-value	0.1139	0.4068
LRT value	4.4911	3.8344
LRT p-value	0.2131	0.2799
Gene diversity	0.4717	0.4044
Heterozygosity	0.5188	0.4310
PIC	0.3737	0.3668

CONCLUSION

Different CSN3 allele and genotype frequencies were found in insemination bulls of Czech Fleckvieh and Holstein cattle. Genotype frequencies of Czech Fleckvieh cattle were AA = 0.391 AB = 0.4887 AE = 0.0075 BB = 0.0902 BE = 0.0226 and EE = 0 and allele frequencies were A = 0.6391 B = 0.3459 E = 0.015. Genetic diversity, heterozygosis and PIC values were following: 0.4717; 0.5188 and 0.3737. Genotype frequencies in Holstein bulls were AA = 0.5517 AB = 0.2759 AE = 0.1207 BB = 0 BE = 0.0345 and EE = 0.0172 and allele frquencies were A = 0.75 B = 0.1552 a E = 0.0948. Genetic diversity, heterozygosis and PIC values were following: 0.4044; 0.4310 and 0.3668. Results of χ^2 tests (Czech Fleckvieh 4.58; Holstein 2.55) show genetical disequilibrium in both examined breeds.

SOUHRN

Genové a genotypové četnosti mléčného proteinu kappa kaseinu u býků v inseminaci plemene českého strakatého a holštýnského

Cílem práce bylo vyhodnocení genových a genotypových četností kappa kaseinu (CSN3) u býků využívaných v inseminaci v České republice u plemene českého strakatého (n = 133) a holštýnského (n = 58). Sledovaní býci byli narozeni v letech 1990–2005. Výpočet byl proveden v programu PowerMarker (Liu K., Muse S. V. 2005). Zjištěné genotypové četnosti u býků českého strakatého plemene byly AA = 0,391 AB = 0,4887 AE = 0,0075 BB = 0,0902 BE = 0,0226 a EE = 0 a genové frekvence A = 0,6391 B = 0,3459 E = 0,015. U býků holštýnského plemene byly genotypové četnosti AA = 0,5517 AB = 0,2759 AE = 0,1207 BB = 0 BE = 0,0345 a EE = 0,0172 a genové četnosti A = 0,75 B = 0,1552 a E = 0,0948. U holštýnského plemen byla zjištěna vyšší četnost alely E a také četnost genotypů s touto alelou. Genetická diverzita, heterozygotnost a hodnota PIC byly vypočítány u býků českého strakatého skotu 0,4717; 0,5188 a 0,3737 a u holštýnských býků 0,4044; 0,4310 a 0,3668. Tyto výsledky ukazují na větší míru genetické variability u býků českého strakatého plemene. Výsledeky χ^2 testu (české strakaté 4,58; holštýnské 2,55) ukazují na genetickou nerovnováhu u býků obou sledovaných plemen využívaných v inseminaci v České republice.

kappa kasein, genové četnosti, genotypové četnosti, býci českého strakatého plemene, býci holštýnského plemene

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