ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Ročník LVI 15 Číslo 1, 2008

POLYMORPHISM OF *CSN3*, *PIT-1*, *LGB* AND ITS IMPACT ON MILK PERFORMANCE TRAITS AT THE CZECH FLECKVIEH AND HOLSTEIN BREED

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Received: August 28, 2007

Abstract

MANGA, I., ŘÍHA, J., VRTKOVÁ, I.: *Polymorphism of CSN3, Pit-1, LGB and its impact on milk performance traits at the Czech Fleckvieh and Holstein breed*. Acta univ. agric. et silvic. Mendel. Brun., 2008, LVI, No. 1, pp. 131–136

The effect of known *CSN3*, *Pit-1* and *LGB* genes single nucleotide polymorphism (SNP) on milk performance traits (milk yield, protein, fat and lactose content, count of somatic cells) at the Czech Fleckvieh and the Holstein cattle breed was estimated. For more exact results, tested cows of each breed came from one source, and were selected into two groups according to their lactation. We tested around 100 animals at the first and 25 at the high (more than five) lactation of both breeds. Our attention was aimed particularly on combined genotypes of favourable alleles. We confirmed the positive tendention of the cheese yield valuable *CSN3/BB* with *Pit-1/AA* combined genotype for high milk production. By estimation of single gene effect, we found out significant association ($P \leq 0.01$) between milk yield (kg) and *Pit-1* genotypes at Holstein cows with the high lactation. This dependence was observed at the Czech Fleckvieh breed too, even it didn't get conclusive value. Among all tested individuals, *CSN3* genotypes significantly influenced milk yield (*AA* > *BB*, $P \leq 0.01$), milk fat (*BB* > *AA*, $P \leq 0.01$) and protein content (*BB* > *AA*, $P \leq 0.01$). The genotypes *AB* and *BB* of the *LGB* gene at Holstein cows were joined with higher milk yield in selected cattle groups opposite to *AA* genotype ($P \leq 0.01$, $P \leq 0.05$). The influence of *LGB* on the somatic cell count in created cattle groups was not confirmed. Estimation of allele and genotype frequency according to lactation stage didn't reveal higher differences at non of tested genes in both breeds.

CSN3, *LGB*, *Pit-1,* SNP polymorphism, milk performance traits

The aim of breeding procedures of the dairy-cow breeds is getting improvement of milk features quality at current high cow's milk production. In terms of longtime breeding proceedings, there have been excellent parameters of cow's milk production accomplished. However, there exist considerable variability in milk performance traits in breeds among particular individuals. This fact could be explained by existing polymorphism of the milk proteins coding genes. Simultaneously, it offers possibilities for other animals selection. Choice of optimal genetic markers in combination with classic breeding procedures represents the most effective tool of livestock breeding, saving a lot of time (Meuwissen, 2003). The set of markers in our work conform for effort of producing milk with high protein content and with optimal technological characteristics for cheese manufacturing (polymorphism *CSN3* and *LGB* genes) at high total milk production (polymorphism *Pit-1*gene) (Renaville, 2005).

The relation of *CSN3* to milk performance traits was evaluated by many authors (Renaville, 2005; Kučerová, 2004). As the most valuable allele is mentioned allele *B*, which is binded with higher percentage of proteins and fat content and also with optimal features for cheese producing. Allele *A* is mostly associated with higher yield of milk, fat and protein. Tsiaras, (2005), explored relations between *CSN3* genotypes and reproduction parameters (lenght of gravidity, age at the first and the second lactation, calving interval) and he didn‘t find any associations with negative influence.

LGB type *B* could serve as a marker for high protein content in milk. Likewise as the type *B* of *CSN3*, *LGB* type *B* is joined with advantage cheese yield features. Milk with genotype *BB* of the *LGB* gene allows obtaining of higher cheese yield in relation to milk with genotype *AA* of the *LGB* (Van den Berg, 1990). Genotype *BB* is often associated with higher percentual content of kaseins and fats in milk, whereas *AA* genotype enhances the level of whey proteins in milk (McLean, 1984; Hill, 1993). Tsiaras, (2005), recently tested these known associations of the LGB, where he detected significant impact of the *LGB* genotypes on milk yield, fat yield, fat content as well as protein yield and content in milk. In addition, testing of *LGB* polymorphism revealed binding with cows resistance to mastitis. Genotypes *BB* and *AB* were in significant relation with lower count of somatic cells (Walawski, 1997), which is an important parameter of milk quality and refers to good health state of dairy udder.

Pit-1 presents specific transcription factor, regulated an expression of GH (growth hormone) in mammals, activates an expression of thyreotropine and prolactine too. Moreover it plays an important role by regulation of cell differentiation and hypophysis proliferation. *Pit-1* together with *GH* present essential genes for adjustment of mammary gland development and for milk secretion (Bauman, 1985; Peel, 1987). Substitution adenine (allele *A*) for guanine (allele *B*) at position 1178 of the *Pit-1* gene (Dierkes *et al.*, 1998) is joined with specific milk performance parameters too. Most often, allele *A* influences milk production of dairycows positively.

The aim of our work was to estimate actual status of screened genetic markers polymorphism at two, the most breeded cattle breeds for milk production in Czech Republic. Important part of our study constituted an evaluation of combined *CSN3*, *Pit-1* genotypes influence on the observed milk performance traits.

MATERIAL AND METHODS

The tested groups of animals consisted of 125 cows of the Czech Fleckvieh breed and equal count of the Holstein breed, both breeds were represented almost entirely by pure breed animals. At the Fleckvieh breed, 100 cows were tested at the first and 25 at the high lactation, at the Holstein breed, 105 cows were tested at the first and 23 at the high lactation.

DNA was isolated from milk using Jet quick blood and cell culture DNA spin kit (Genomed). To detect the genes polymorphism, we used PCR-RFLP method: for *CSN3* analysis described by Soria *et al.*, (2003), for *Pit-1* described by Renaville *et al.*, (2005) and for *LGB* described by Strzalkowska *et. al.*, (2002). Results of testing genes polymorphism were evaluated in relation to follows milk performance parameters: milk yield (kg), percentage of fat, proteins and lactose, and count of somatic cells (thous/ml).

Acquired results of analysis were processed in SAS 8.2 programme. ANOVA and GLM collections of methods were used for calculations of – 1) differences among groups caused by lactation (one-way F-test); 2) differences among groups caused by genotype (Tukey post-hoc test); 3) differences among groups caused by genotype and lactation. Following model was used for GLM analysis:

$$x_{ijk} = m + a_j + b_k + e_{ijk},$$

where:

x_{ijk} is a value of milk parametr for individual *i* with genotype *j* in lactation *k*,
m is a mean value of parametr,
a_j is an effect of *j*-th genotype,
b_k is an effect of *k*-th lactation,
e_{ijk} is a random error.

RESULTS AND DISCUSSION

We estimated the relative genotypes and allele frequencies of tested markers at both breeds as shown in Table I, II and III. Acquired frequencies of the *CSN3* gene (Table I.) are in agreement with findings of other authors (Kučerová, 2004). Clear milk breed as Holstein featured with lower frequency of valuable allele *B* opposite to the combined meat-milk performance Czech Fleckvieh breed. Among particular lactations, there haven’t been significant associations in allele frequency differences. We noted high significant associations in milk yield, milk fat and protein content by analysis of all lactations according to *CSN3* genotypes regardless of the breed classification. Here, the allele *A* positive influenced the milk yield ($P \leq 0.01$), as well as the allele *B* increased the fat ($P \leq 0.01$) and protein ($P \leq 0.01$) content. These finding also confirm findings in other studies.

The estimation of effect combined *Pit-1* and *CSN3* genotypes on milk yield lended outcomes, which are viewable in Table IV. The results indicate, that the impact of allele *A* in both genes on milk yield is viewable at the combined *Pit-1*/*CSN3* genotypes too. By comparison detected evidential associations among combined genotypes, the genotype *BB*/*BB* featured always with lower milk production at the Holstein breed at the first lactation. Even if the desirable *AA*/*BB* genotype, by reason of deficient count of animals wearing this genotype, couldn’t be evaluated even at one breed, but the similar genotype *AB*/*BB* had equally positive effect on elevation milk production. The situation at the Fleckvieh breed was the same, though just the *BB*/*AA* combined genotypes at the first lactation produced significantly more milk opposite to *BB*/*BB* genotypes. Renaville, (2005), detected effect +273kg on milk yield/ per lactation/per dairycow of the *AA*/*BB* combined genotypes in comparison to *BB*/*AA* genotypes at tested Holstein breed. This is in agreement with findings of our work. Hence, selection of combined *Pit-1*/*CSN3* genotypes *AA*/*BB* (*AB*/*BB*) at the dairycows breeds should afford a way, how to ensure production of milk with valuable *BB*/*CSN3* genotypes at simultaneously high milk production. Unfortunately, effect of combined genotypes on milk performance traits of cows tested at the high lactation can’t be evaluated considering their low count.

I: *CSN3 gene: comparison of relative genotype and allelic frequency in breeds tested at the first and the high lactation*

gene *CSN3*	lactation	n	genotype frequency			allele frequency	
			AA	AB	BB	A	B
Czech Fleckvieh	first	111	0.26	0.58	0.15	0.55	0.46
	high	30	0.37	0.33	0.30	0.53	0.47
	all	141	0.28	0.53	0.18	0.55	0.45
Czech Holstein	first	67	0.60	0.30	0.10	0.75	0.25
	high	14	0.43	0.57	0.00	0.71	0.29
	all	81	0.57	0.35	0.09	0.74	0.26

II: *Pit-1 gene: comparison of relative genotype and allelic frequency in breeds tested at the first and the high lactation*

gene *Pit1*	lactation	n	genotype frequency			allele frequency	
			AA	AB	BB	A	B
Czech Fleckvieh	first	104	0.02	0.14	0.85	0.09	0.91
	high	31	0.10	0.07	0.84	0.13	0.87
	all	135	0.04	0.12	0.84	0.10	0.90
Czech Holstein	first	66	0.03	0.27	0.70	0.17	0.83
	high	14	0.00	0.36	0.64	0.18	0.82
	all	80	0.03	0.29	0.69	0.17	0.83

III: *LGB gene: comparison of relative genotype and allelic frequency in breeds tested at the first and the high lactation*

gen *LGB*	lactation	n	genotype frequency			allele frequency	
			AA	AB	BB	A	B
Czech Fleckvieh	first	106	0.07	0.62	0.31	0.38	0.62
	high	31	0.13	0.45	0.42	0.36	0.65
	all	137	0.08	0.58	0.34	0.37	0.63
Czech Holstein	first	67	0.13	0.70	0.16	0.49	0.52
	high	14	0.14	0.64	0.21	0.46	0.54
	all	81	0.14	0.69	0.17	0.48	0.52

IV: *Estimation of combined Pit-1 and CSN3 genotypes influence on milk yield of the Czech Fleckvieh and Holstein cows tested at the first lactation*

genotypes	to: *Pit-1 BB/ CSN3 BB*	p	breed*	lact.
Pit-1 AB/ CSN3 BB	>	0.037	H	1
Pit-1 BB/ CSN3 AB	>	0.005	H	1
Pit-1 AB/ CSN3 AB	>	0.006	H	1
Pit-1 AB/ CSN3 AA	>	0.01	H	1
Pit-1 BB/ CSN3 AA	>	0.01	H	1
Pit-1 BB/ CSN3 AA	>	0.05	F	1

* H – Czech Holstein, F – Czech Fleckvieh breed

By evaluation of individual *Pit-1* polymorphism impact on observed milk performance parameters at the Holstein breed, we found out, that cows tested at the high lactation with *AB* genotype produced significantly more milk ($P \leq 0.01$) than cows with *BB* genotype. This dependence was detectable at the Fleckvieh breed with the high lactation too, while without the evidential significance. These shown data are in agreement with findings of many authors (Renaville, 1997; Vargas, 2004). Among *Pit-1* genotypes, at the dairycows tested at the first lactation we didn't note any significant associations as compared with combined *Pit-1/CSN3* genotypes. Genotype *AB* and its positive action to milk yield as the *BB* genotype with reducing impact on milk yield showed itself till at the high lactation. The parameters of milk performance traits at the first calving cows are an object of high economic interest. The physiology differences, different running of pre and post gravidity period of these cows were probably so much high, that individual impact of *Pit-1* polymorphism couldn't be displayed. However, combined influence of both *Pit-1* and *CSN3* markers on milk yield (allele *A*) demonstrated as well at cows tested at the first lactation.

At the Czech Fleckvieh breed at the high lactation, animals with genotype *BB* produced significantly more milk ($P \leq 0.01$) as the same genotypes at the first lactation. This shows evidence of known lower milk production of the first calving cows. Testing of *Pit-1* polymorphism at both breeds didn't reveal any significant relation to fat and protein content.

Relative frequencies of *Pit-1* genotypes and alleles are shown in Table II. The values of frequency allele *A* at the Fleckvieh cattle were rightly lower than at the Holstein cattle. The frequency of allele *A* was slightly lower at both breeds in comparison with works of other authors. At cows of both breeds tested at the high lactation, the reason should be lower animals count in this group. Anyway, this observed frequency didn't differ much from cows at the first lactation with sufficient count of screened individuals, where the relative frequency of allele reached similar values. Among *Pit-1* genotypes, *BB* genotypes have the largest abundance at both breeds. In selected cattle groups according to lactation, there haven't been distinct differences so in allele frequency, as in genotype frequency.

The detected relative frequencies of *LGB* alleles and genotypes are shown in Table III. The frequency of allele *B*, so as the frequency of *BB* genotypes was a few higher at the Fleckvieh breed opposite to the Holstein breed. The frequencies of alleles and genotypes didn'n differ much again in created groups according to degree of lactation at the both breeds. The studies about *LGB* polymorphism impact on milk performance traits are considerably inconsistent. E.g. based on Bovenhuis *et al.*, (1992) the allele *A* enhanced the milk yield, based on other authors decreased, farther based on Hill (1993) the allele *A* enhanced the milk fat content, based on the other authors (Hill, 1993; Tsiaras, 2005) decreased. In our study, we found out significant higher milk yield at *AB* opposite to *AA* genotypes at the first lactation ($P \leq 0.01$) so as at the high lactation ($P \leq 0.01$) of the Holstein cattle. At the same breed, *BB* genotypes produced also more milk opposite to *AA* genotypes with statistical evidence $P \leq 0.05$ at the first lactation, the equal trend at the high lactation didn't reach significant evidence. Polymorphism of *LGB* didn't influence the milk fat content. Holstein cows at the high lactation with *AA* genotype produced milk with significantly higher fat content than cows with the same genotype at the first lactation ($P \leq 0.05$). Likewise, the protein content in milk was higher at the high lactation opposite to the first lactation at the *AA* genotype ($P \leq 0.05$). These findings suggest, that in cows at the high lactation as compared to cows at the first lactation with the same *LGB* genotype alter not just the quantitative production parameters, but the quality parameters like milk fat and protein content too. The influence of *LGB* on the somatic cell count in created cattle groups at the Holstein cattle was not confirmed. Anyway, cows at the high lactation featured with higher somatic cells count at the same genotypes ($P \leq 0.01$ for *AB* and $P \leq 0.05$ for *BB*) so as at different genotypes (*AB* > *AA*, $P \leq 0.01$, *AB* > *BB*, $P \leq 0.01$) opposite to cows at the first lactation.

At the Fleckvieh breed, we noted similar trend among content of milk fat as by Holstein cattle. Further, genotype *AB* by cows at the high lactation featured with higher count of somatic cells opposite to the same genotype at the first lactation ($P \leq 0.01$). These findings are consistent with knowledge, that the full-grown cows featured with higher somatic cells count opposite to the young cattles (Samoré, 2003).

CONCLUSIONS

Observed higher frequencies of alleles *B/CSN3*, *B/LGB* and lower *A/Pit-1* allele frequencies of the Czech Fleckvieh cattle correspond with its zootechnic and production characterization. In contrast to The Czech Fleckvieh cattle, observed higher values of allele frequency *A/CSN3*, *A/Pit-1* and lower *B/LGB* frequency at the Holstein breed truly characterise and copy differencies in breed targets both breeds. Noted differencies in allele and genotype frequency by both breeds reached surprisingly just a low value. As The Czech Fleckvieh, so the Czech Holstein holded almost the same rate of allele frequency tested genes in groups according to lactation, frequency of genotypes shifted a little. Polymorphism of tested genes influences detectably some milk performance traits: Allele *A* of the *Pit-1* gene had a positive effect on milk yield in individual, so as in combined Pit-1/ CSN3 genotypes by both breeds. Therefore, selection for cheese yield valuable *CSN3/BB* with *Pit-1/AA* combined genotypes could be a perspective selection procedure. The allele *B* of the *LGB* gene was binded with higher milk yield opposite to allele *A* in selected cattle groups the Czech Holstein. We confirm the *CSN3* polymorphism effect to milk yield and protein content at both breeds. Cows at the high lactation stage featured with higher somatic cell count opposite to cows at the first lactation. At both breeds, the *LGB* polymorphism influence on the somatic cell count in created cattle groups was not confirmed.

SOUHRN

Polymorfismus genů *CSN3*, *Pit-1*, *LGB* u plemen český strakatý skot a český holštýn a jeho vliv na parametry mléčné užitkovosti

V práci byl stanoven efekt známých SNP polymorfismů genů *CSN3*, *Pit-1* a *LGB* na parametry mléčné užitkovosti (produkce mléka, obsah tuku, proteinu a laktózy, počet somatických buněk) u 125 dojnic českého strakatého plemene a u 128 dojnic plemena český holštýn. Jedinci zkoumaných plemen pocházeli z jednoho chovu a kvůli exaktnějšímu vyhodnocení asociací byli testováni ve skupinách dle stupně laktace (skupina na první laktaci a skupina na vyšší laktaci (> 5)). Identifikace genotypů byla provedena metodou PCR–RFLP. Na zjištění statisticky významných asociací mezi genotypy a sledovanými znaky byl využit program SAS 8.2 a komplex metod ANOVA. Testováním polymorfismu *CSN3* bez ohledu na plemennou příslušnost a stupeň laktace byl zjištěn jeho významný vliv ($P \leq 0{,}01$) na celkovou produkci mléka (*AA* > *AB* > *BB*), na obsah mléčného tuku (*BB* > *AA*, $P \leq 0{,}01$) a na obsah mléčných proteinů (*BB* > *AA*, $P \leq 0{,}01$). Genotypy *AB*/*Pit-1* byly v asociaci s vyšší produkcí mléka ($P \leq 0{,}01$) v porovnání s genotypy *BB*/*Pit-1* u dojnic holštýnského plemene testovaného při vysoké laktaci. Podobná asociace bez statistického významu byla zaznamenána také u strakatého plemene. Průkazný pozitivní vliv alely *A* na produkci mléka při první laktaci byl evidentní také v kombinovaných *Pit-1*/*CSN3* genotypech, zejména u plemena holštýn. Selekce na sýrařsky žádoucí *CSN3*/*BB* genotypy v kombinaci s *Pit-1*/*AA* genotypy proto může představovat perspektivní selekční metodu. U jedinců s vysokým stupněm laktace z důvodu nízkého počtu jednotlivých kombinací genotypů efekt nebyl potvrzen. Genotypy *AB*/*LGB* produkovaly v porovnání s genotypy *AA*/*LGB* průkazně více mléka u obou testovaných skupin plemene český holštýn ($P \leq 0{,}01$), (podobně *BB* > *AA* ($P \leq 0{,}05$) při první laktaci). Příznivý vliv alely *B* na produkci mléka byl bez průkazného rozdílu viditelný rovněž u dojnic strakatého plemene. Vliv polymorfismu *LGB* na obsah somatických buněk v mléce nebyl nalezen.

Vyhodnocením frekvence alel a genotypů všech tří genů mezi skupinami dojnic dle stupně laktace nebyly zjištěny významné rozdíly ani u jednoho z plemen.

CSN3, *Pit-1*, *LGB*, SNP polymorfismus, parametry mléčné užitkovosti

This research was supported by the Czech Science Foundation project No. 523/03/H076 and Ministry of Agriculture of the Czech Republic 1G58073.
Thanks are offered to LAMGen company for samples providing to this work.

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