

Effects of 5 SNPs on daughters' milk performance traits produced by Ukrainian dairy sires

Olena Fedota¹, Nina Lysenko², Larysa Mitiohlo³, Sergiy Ruban⁴

¹ V. N. Karazin Kharkiv National University, Svobody Sq., 4, Kharkiv, 61022, Ukraine
<http://orcid.org/0000-0001-9659-383X>, e-mail: amsfedota@gmail.com, Tel.: +38-067-961-77-11

² Kharkiv National Medical University, Nauky Avenue, 4, Kharkiv, 61022, Ukraine
<https://orcid.org/0000-0002-3388-0038>, e-mail: n.g.lysenko@gmail.com, Tel.: +38-099-640-90-23

³ Research farm "Nyva", Institute of Animal Breeding and Genetics nd. a. M.V. Zubets of NAAN, State owned enterprise, vul. Sadova, 1, Hristinivka, Hristinivskiy dist, Cherkas'ka oblast, 20009, Ukraine

⁴ National University of Life and Environmental Sciences of Ukraine, Heroiv Oborony Str., 15, Kyiv, 03041, Ukraine

Received: 14.02.2018. Accepted: 20.03.2018

The aim of the study was to estimate the effects of the CAPN316, CAST282, GH L127V, GHR F279Y and A257G on milk yield, fat and protein yield and content on daughters produced by dairy sires. 24 dairy sires were 100% Holstein (14), 100% Brown Swiss (5) and Holstein mixed with another dairy breeds (5). Analyses included data on 32733 daughters for 2014-2017 years. Molecular genetic analysis was performed by PCR-RFLP. Statistical analysis revealed that population was on Hardy-Weinberg equilibrium. SNPs F279Y and A257G were not in linkage disequilibrium ($r^2 = 0.029$, $D' = 0.074$), because linkage of certain alleles was observed only in 7.4% of cases (D'). C-allele of SNP CAPN316 was strongly associated with protein content, despite C-allele of SNP CAST282 showed negative association with milk, fat and protein yield. Daughter milk performance traits (DMPTs) for SNP CAPN316 corresponded to CC>CG>GG pattern, except variations in milk and fat yield (kg). For SNP CAST282 Milk, fat and protein yield (kg) were significantly higher for CG than for CC genotype. C-allele for SNP L127V showed significant association and differences between genotypes with DYDs for fat yield (kg) – 20.45 ± 5.53 ($P < 0.01$) and content (%) – 0.13 ± 0.06 ($P < 0.05$), T-allele for SNP F279Y was not significantly associated with any trait studied and A-allele for SNP A257G significantly associated with milk and fat yield (kg) for DMPTs/DYDs 1988.33 ± 419.93 / -269.69 ± 89.92 ($P < 0.01$) and 84.30 ± 15.64 / -8.33 ± 3.87 ($P < 0.05$). Results of this study reveal and support known advantages of SNPs studied in panels and selection programs.

Keywords: dairy sires; milk production traits; genotype; CAPN316; CAST282; L127V; F279Y; A257G

Вплив 5 SNPs на характеристики молока дочок бугаїв порід молочного напрямку

Олена Федота¹, Ніна Лисенко², Лариса Мітіогло³, Сергій Рубан⁴

¹ Харківський національний університет імені В. Н. Каразіна, майдан Свободи 4, 61022, Харків, Україна
<http://orcid.org/0000-0001-9659-383X>, e-mail: amsfedota@gmail.com, Тел.: +38-067-961-77-11

² Харківський національний медичний університет, проспект Науки, 4, 61022, Харків, Україна
<https://orcid.org/0000-0002-3388-0038>, e-mail: n.g.lysenko@gmail.com, Тел.: +38-099-640-90-23

³ Дослідне господарство «Нива» Інституту розведення і генетики тварин імені М.В. Зубця НААН України, Державне підприємство, вул. Садова, 1, с. Христинівка, Христинівський р-н, Черкаська обл., 20009, Україна

⁴ Національний університет біоресурсів і природокористування України, вулиця Героїв Оборони, 15, Київ, 03041, Україна

Метою дослідження було оцінити ефекти CAPN316, CAST282, GH L127V, GHR F279Y та A257G на молочність, жирномолочність та білковомолочність та вміст жиру та білку у дочок бугаїв порід молочного напрямку. 24 бугаї належали до порід: 100% голштинська (14), 100% швіцька (5) та голштинської породи, мішаної з іншими породами молочного напрямку (5). Аналіз включав дані 32733 доньок протягом 2014-2017 років. Молекулярно-генетичний аналіз проводили за допомогою ПЛР-ПДРФ. Статистичний аналіз показав, що популяція знаходилась у рівновазі за Харді-Вайнбергом. SNP F279Y та A257G не демонстрували нерівновагу за зчепленням ($r^2 = 0,029$, $D' = 0,074$), оскільки зв'язок певних алелей спостерігався лише у 7,4% випадків (D'). С-алель SNP CAPN316 характеризувався високою асоціацією із

вмістом білку, незважаючи на те, що *C*-алелі SNP *CAST282* показали негативну асоціацію з молочністю, жирномолочністю та білковомолочністю. Молочні характеристики дочок (DMPTs) для SNP *CAPN316* відповідає закономірності *CC* > *CG* > *GG*, за винятком молочності та жирномолочності (кг). За SNP *CAST282* молочність, жирномолочність та білковомолочність (кг) були значно вищими для генотипу *CG*, ніж для *CC*. *C*-аллель за SNP *L127V* показав значну асоціацію та різницю між генотипами з DYDs для жирномолочності (кг) - $20,45 \pm 5,53$ ($P < 0,01$) та вмісту жиру (%) - $0,13 \pm 0,06$ ($P < 0,05$), *T*-аллель за SNP *F279V* не був значно асоційовим з будь-якою досліджуваною рисою, але *A*-аллель за SNP *A257G* значно асоціювався з молочністю та жирномолочністю (кг) для DMPTs/DYDs $1988,33 \pm 419,93$ / $-269,69 \pm 89,92$ ($P < 0,01$) та $84,30 \pm 15,64$ / $-8,33 \pm 3,87$ ($P < 0,05$). Результати цього дослідження виявляють та підтверджують відомі переваги досліджуваних SNP для використання в панелях та програмах відбору.

Ключові слова: бугії молочних порід; молочні характеристики; генотип; *CAPN316*; *CAST282*; *L127V*; *F279V*; *A257G*

Introduction

The study of SNPs in candidate genes affecting productive and reproductive processes is actual for explaining genetic variation, for understanding their contribution to vital functions and inclusion them into SNP panels used in animal evaluation and breeding. According Cochran et al. (2013), different types of SNPs were evaluated for their relationship to reproductive animal traits: SNPs previously reported to be associated with reproductive traits of dairy or beef cattle or physically close to genetic markers for reproduction, SNPs in genes that are well known to be involved in reproductive processes, and SNPs in genes reported to be differentially expressed between physiological conditions in a variety of tissues associated in reproductive function. As the researchers concluded incorporation of candidate gene SNPs into genomic tests for reproduction would allow selection of causative SNPs or SNPs physically more close to causative SNPs. Such an approach has been successful for improving ability to detect genomic associations with disease (Amos et al., 2011; Cochran et al., 2013). Also an important point in the analysis of gene networks is a loss of information from multiple single nucleotide polymorphisms located within or close to the same gene, ignoring information on linkage disequilibrium and validation of the obtained gene network (Suchocki et al., 2016).

It is known that many female fertility traits in dairy cattle show antagonistic genetic correlations with milk production traits but with low or moderate correlations. This implies that simultaneous genetic selection for increased milk yield and reproductive performance is possible. Simultaneous breeding for both productive and fertile cows would benefit substantially from knowing the genetic and physiological links between production and health to disentangle the effects on these traits (Iso-Touru, et al., 2016; Berry et al., 2016; Aliloo et al., 2015). Given that dairy cattle reproduction is usually performed with sperm produced by a limited number of sires, we need to provide early evaluation of sires' quality based on genotyping of markers for daughters' milk performance.

The calpain system, consisting of μ -calpain, m-calpain and their inhibitor calpastatin has a number of different roles in cells, including but not limited to "remodeling" of cytoskeletal attachments to the plasma membrane during cell fusion and cell motility, proteolytic modification of molecules in signal transduction pathways, degradation of enzymes controlling progression through the cell cycle, regulation of gene expression, substrate degradation in some apoptotic pathways, and an involvement in long-term potentiation (Goll et al., 2003). Due to post mortal activity of calpain system in a muscle tissue, resulting in cytoskeleton fibers degradation, SNPs of *CAPN1* and *CAST* genes are used as markers for meat tenderness in beef cattle (Schenkel et al., 2006; Gill et al., 2009). In dairy cattle *CAPN1* is primary associated with reproductive characteristics – the ability to ovulate prior to weaning and post-partum anoestrus interval (Collis et al., 2012), and the *CAST* gene is associated with daughter pregnancy rate (Ortega et al., 2016). Given the association of some SNPs in these genes with udder size, it is possible to assume the relation to quantitative and qualitative parameters of milk. Considering that traditionally dairy sires are evaluated by daughter productivity and presence of calpain system enzymes in sperm that affects semen quality traits (Cui et al., 2016), we consider it is appropriate to evaluate the effect of SNPs *CAPN316* and *CAST282* in dairy sires on their daughter milk productivity performance.

Growth hormone (GH) interacting with receptor (GHR) is involved in regulation of animal growth and development, it affects the quality parameters of milk and fertility of cows via enhancing lipolysis in adipose tissue (Balogh et al., 2009; Hadi et al., 2015), therefore GH and GHR genes can be considered as standard markers of both dairy and beef cattle productivity. SNPs *L127V* of the *GH* gene, *F279Y* and *A257G* of the *GHR* gene (Blott et al., 2003; Viitala et al., 2006) are associated with productive characteristics as milk yield, fat and protein content and somatic cell count, age of reproductive period onset, calving interval, predisposition to mastitis, birth weight, body weight gain and constitution (Hadi et al., 2015; Rahmatalla et al., 2011; Olenski et al., 2010). Additionally, male gonad and accessory organs are sites of both GH production and action (Hull and Harvey, 2014). Therefore, our aim was to analyze the effect of SNPs *CAPN316*, *CAST282*, *L127V*, *F279Y* and *A257G* on breeding values of Ukrainian dairy sires for milk production traits and milk performance traits of their daughters.

Materials and methods

The study included 24 dairy sires of 100% Holstein (14), 100% Brown Swiss (5) and Holstein mixed with Simmental or Montbéliarde or Black-and-White or both (5) breeds. Analyses included data on 32733 daughters produced by analyzed sires during 2014-2017 years. Daughter yield deviation (DYD) is a standard measure of animals' genetic merits in routine genetic evaluations worldwide (Szyda et al., 2008). DYDs and characteristics of the daughters' milk performance traits included milk (MY, kg), fat (FY, kg) and protein yield (PY, kg), fat (FP, %) and protein percentage (PP, %). These data were withdrawn from farm records and Catalogue of Dairy and Dual Purpose Sires for Breeding Stock Reproduction (2014-2017), <http://www.animalbreedingcenter.org.ua/catalog>.

DNA of sires was isolated from whole blood or from frozen sperm. Analyses were performed by PCR/RFLP techniques, published in our previous papers (Ruban et al., 2017, Fedota et al., 2017) using regimens published by Miquel et al. (2009) and Komisarek et al. (2011). Whereas F279Y and A257G are the SNPs in the same gene GHR, located in the 8th and 10th exons, separated with approximately 18 kb (Fedota et al., 2017), linkage disequilibrium (r^2 ; Hill and Robertson 1968) was calculated between their pairwise combination. The association between each SNP and all DYDs and daughters' milk parameters of Ukrainian dairy sires for milk production traits was quantified using weighted linear models mixed in R (Maechler et al., 2013) included year of birth and percent Holstein of the individual sire as fixed effects and genotype as a continuous variable. For comparison between two and more groups we used Student's t-test and ANOVA. Pearson's Chi-square test was applied to prove whether a Hardy-Weinberg genetic equilibrium was fulfilled in the population. Allele substitution effects were estimated by regressing the number of copies of SNP allele against the analyzed DYDs or daughters' milk performance traits. $P \leq 0.05$ indicated significant difference.

Results

Descriptive data for DYDs and daughters' milk performance traits (DMPTs) by year are summarized in Table 1. Total number of daughters depends on commercial availability or marketing strategy for given sires, although sires number per year was determined by citations in a Catalogue. The variability (CV, %) is higher for DYDs rather than for DMPTs, being the highest for fat and protein content due to wide range of variation for small numbers.

Table 1. Basic statistics for DYD and DMPTs of Ukrainian dairy sires by year.

	N sires	N daughters	\bar{x}	s_x	CV, %	Minimum	Maximum
2014							
DMPTs							
Milk yield (kg)	16	2316	8407.00	513.16	6.10	5487	11488
Fat yield (kg)	16	2316	335.19	19.27	5.75	205	454
Protein yield (kg)	14	2218	295.64	13.73	4.64	190	360
Fat content (%)	16	2316	4.01	0.10	2.57	3.56	5.24
Protein content (%)	14	2218	3.39	0.09	2.65	3.09	4.39
BBV (pt)	17	2433	1072.06	54.95	5.13	758	1690
DYDs							
Milk yield (kg)	17	2433	942.94	88.13	9.35	309	1667
Fat yield (kg)	17	2433	34.94	4.02	11.51	3	64
Protein yield (kg)	15	2335	32.93	2.94	8.94	18	60
Fat content (%)	17	2433	-0.03	0.04	-	-0.57	0.16
Protein content (%)	15	2335	0.01	0.02	-	-0.25	0.14
2015							
DMPTs							
Milk yield (kg)	9	10667	7051.11	790.40	11.21	4461	11854
Fat yield (kg)	9	10667	266.33	29.71	11.15	167	429
Protein yield (kg)	6	10549	260.83	26.46	10.14	196	358
Fat content (%)	9	10667	3.78	0.04	1.03	3.62	4.04
Protein content (%)	6	10549	3.19	0.08	2.45	3.02	3.54
BBV (pt)	20	19077	823.95	87.85	10.66	250	1646
DYDs							
Milk yield (kg)	20	19077	827.10	114.57	13.85	159	1837
Fat yield (kg)	20	19077	32.70	4.32	13.22	-4	69
Protein yield (kg)	17	18959	28.53	3.85	13.50	8	64
Fat content (%)	20	19077	0.02	0.04	-	-0.48	0.29
Protein content (%)	17	18959	0.04	0.03	-	-0.19	0.29
2016							
DMPTs							
Milk yield (kg)	6	1986	6840.67	833.24	12.18	4461	9529
Fat yield (kg)	6	1986	265.50	36.96	13.92	167	402
Protein yield (kg)	4	983	260	29.17	11.22	201	339
Fat content (%)	6	1986	3.85	0.08	2	3.71	4.22
Protein content (%)	4	983	3.23	0.11	3.45	3.09	3.56
BBV (pt)	14	4786	804.21	114.98	14.30	130	1322
DYDs							
Milk yield (kg)	14	4786	867.29	140.13	16.16	31	1837
Fat yield (kg)	14	4786	36.79	4.24	11.52	3	56
Protein yield (kg)	12	4688	28.75	4.70	16.35	10	52
Fat content (%)	14	4786	0.06	0.06	-	-0.51	0.32
Protein content (%)	12	4688	0.05	0.04	-	-0.24	0.29
2017							
DMPTs							
Milk yield (kg)	10	3571	7251.20	603.50	8.32	4461	9529
Fat yield (kg)	10	3571	281.40	26.25	9.33	167	402
Protein yield (kg)	7	3453	274.86	17.29	6.29	211	339
Fat content (%)	10	3571	3.85	0.05	1.32	3.71	4.22
Protein content (%)	7	3453	3.29	0.07	2.21	3.11	3.56
BBV (pt)	17	6437	839	95.70	11.41	158	1894
DYDs							
Milk yield (kg)	17	6437	851.59	126.95	14.91	28	1852
Fat yield (kg)	17	6437	35	4.55	13	-1	75
Protein yield (kg)	14	6319	29.71	4.44	14.94	10	68
Fat content (%)	17	6437	0.01	0.05	-	-0.51	0.31
Protein content (%)	14	6319	0.04	0.04	-	-0.24	0.33

Note: DMPTs – daughter milk performance traits, BBV – bulls' breeding value by national Ukrainian scale, DYDs – daughters' yield deviation.

The allele and genotype frequencies for all SNPs studied are shown in Table 2. Expected genotypic frequencies were similar to the observed ones suggesting that genotype distributions were in the Hardy-Weinberg equilibrium.

Table 2. Genotype frequencies (GF), allele frequencies (AF) and significance of deviation for Hardy-Weinberg equilibrium (HWE) for SNPs *CAPN316*, *CAST282*, *L127V*, *F279Y* and *A257G*

SNP	Genotype	N	GF (%)	AF (%)	HWE
<i>CAPN316</i>	CC	3	0.125	C = 0.355	0.001
	CG	11	0.458	G = 0.645	
	GG	10	0.417		
<i>CAST282</i>	CC	10	0.417	C = 0.667	0.195
	CG	12	0.500	G = 0.333	
	GG	2	0.083		
<i>L127V</i>	CC	1	0.111	C = 0.445	0.576
	CG	6	0.667	G = 0.555	
	GG	2	0.222		
<i>F279Y</i>	TT	7	0.636	T = 0.727	1.482
	TA	2	0.182	A = 0.273	
	AA	2	0.182		
<i>A257G</i>	AA	8	0.667	A = 0.792	0.298
	AG	3	0.250	G = 0.208	
	GG	1	0.083		

Considering that differences between groups was not significant (ANOVA), we performed further analysis for pooled data. There were a number of strong associations between DMPTs, DYDs and SNPs (Table 3).

Table 3. Estimated allele substitution effect (B±SE_B) between SNPs *CAPN316*, *CAST282*, *L127V*, *F279Y*, *A257G* and daughter milk performance traits and DYDs.

SNP	<i>CAPN316</i>	<i>CAST282</i>	<i>L127V</i>	<i>F279Y</i>	<i>A257G</i>
Allele substitution	C>G	C>G	C>G	T>A	A>G
DMPTs					
	-	-	-	-399.38±749.37	1988.33±419.93***
Milk yield (kg)	327.25±491.1				
	1				
Fat yield (kg)	-4.03±20.04	-	-	-12.16±28.97	84.30±15.64***
Protein yield (kg)	14.70±17.79	-	-	17.58±18.63	-
Fat content (%)	0.13±0.07 [†]	-	-	0.07±0.15	0.14±0.09
Protein content (%)	0.24±0.08**	-	-	0.19±0.13	-
BBV (pt)	-5.46±267.25	-338.32±340.73	68.19±147.31	178.18± 557.96	396.62±382.31
DYDs					
Milk yield (kg)	-60.49±78.08	37.63±100.60	223.89±181.7	-195.79±116.39 [†]	-269.69±89.92**
			2		
Fat yield (kg)	-1.76±3.18	4.88±4.05	20.45±5.53**	-8.59±4.64 [†]	-8.33±3.87*
Protein yield (kg)	-2.97±3.19	-1.07±3.75	3.92±6.22	-5.26±3.96	-2.17±4.75
Fat content (%)	-0.01±0.03	0.05±0.04	0.13±0.06*	0.01±0.04	-0.01±0.03
Protein content (%)	0.01±0.02	0.01±0.03	-0.01±0.03	0.04±0.02 [†]	-0.01±0.02

Note: Significance of difference from zero [†]P < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001.

CAPN316 and *CAST282*

C-alleles of SNPs *CAPN316* (calpain gene) and *CAST282* (calpastatin gene) are associated with meat tenderness (Gill et al., 2009). C-allele frequency for *CAPN316* was lower, alternatively to *CAST282*. C-allele of SNP *CAPN316* was strongly associated with protein content, despite C-allele of SNP *CAST282* showed negative association with milk, fat and protein yield (Table 3). Tables 4 and 5 summarize the differences between daughter milk performance traits and DYDs by *CAST316* and *CAST282* genotypes. DMPT for SNP *CAPN316* corresponded to CC>CG>GG pattern, except variations in milk and fat yield (kg). However, significant increased fat yield for genotype CG could be determined by significant increased milk yield, where R^2 did not exceed 30%. Significant differences in protein content (%) were observed. DYDs were significant or close to significance level for fat content (%) and

yield (kg), respectively. Data for GG-genotype for *CAST282* on DMPT were not provided, therefore we compared only two groups and could not perform association analyses. Milk, fat and protein yield (kg) were significantly higher for CG than for CC genotype. All DYDs traits were similar between genotypes and showed high variation exceeding 10-15% for some genotypes with $R^2 < 5\%$.

Table 4. DMPTs and DYDs by *CAPN316* genotype.

Genotype	CC	CG	GG	P	R ²
DMPTs					
Milk yield (kg)	5103.40±434.04	8641.06±279.70	7248.44±568.77	0.001	0.294
Fat yield (kg)	210.80±35.08	340.28±10.21	277.11±22.96	0.003	0.264
Protein yield (kg)	292.00	285.67±9.38	269.50±20.70	0.713	0.024
Fat content (%)	4.04±0.30	3.95±0.05	3.80±0.04	0.171	0.089
Protein content (%)	4.39	3.30±0.04	3.21±0.05	0.001	0.574
BBV (pt)	755.60±127.89	913.97±65.99	863.28±77.26	0.517	0.021
DYDs					
Milk yield (kg)	822.10±724.08	293.20±59.28	646.00±389.93	0.529	0.021
Fat yield (kg)	760.70±158.66	805.30±82.20	875.96±81.31	N/A	1.000
Protein yield (kg)	39.70±4.91	28.73±3.38	38.64±3.27	0.067	0.084
Fat content (%)	20.50±3.86	30.63±2.54	29.95±3.98	0.312	0.044
Protein content (%)	0.14±0.01	-0.06±0.04	0.07±0.02	0.0039	0.164
Protein content (%)	0.06±0.02	0.04±0.02	0.05±0.02	0.842	0.007

Table 5. DMPT and DYDs by *CAST282* genotype.

Genotype	CC	CG	GG	P	R ²
DMPTs					
Milk yield (kg)	6939.14±378.2	8290.30±515.09	-	0.040	-
Fat yield (kg)	267.62±16.16	327.35±19.84	-	0.024	-
Protein yield (kg)	249.80±12.16	307.56±10.92	-	0.001	-
Fat content (%)	3.83±0.04	3.96±0.08	-	0.165	-
Protein content (%)	3.23±0.04	3.37±0.08	-	0.150	-
BBV (pt)	833.23±83.39	914.59±55.89	526.50±56.50	0.299	0.038
DYDs					
Milk yield (kg)	222.76±62.12	722.49±313.16	110.00±6.00	0.400	0.029
Fat yield (kg)	828.88±81.93	840.49±75.77	508.00±63.00	0.584	0.017
Protein yield (kg)	37.15±2.72	32.65±3.36	25.50±8.50	0.485	0.023
Fat content (%)	27.60±3.70	30.82±2.49	21.00±3.00	0.552	0.023
Protein content (%)	0.06±0.02	-0.01±0.04	0.06±0.06	0.362	0.032
Protein content (%)	0.04±0.02	0.04±0.02	0.04±0.02	0.997	0.001

L127V, *F279Y* and *A257G*

The analysis revealed high frequency for "wild" type alleles for all three polymorphic variants, these are C-allele for *L127V* (growth hormone gene), T-allele for *F279Y* and A-allele for *A257G* (growth hormone receptor gene). Absent data on CC genotype for *L127V* prohibited to perform association analyses for DMPT. C-allele for *L127V* showed significant association and differences between genotypes with DYDs for fat yield (kg) and content (%) (Table 6), T-allele for *F279Y* was not significantly associated with any trait studied and A-allele for *A257G* significantly associated with milk and fat yield (kg) for DMPT and DYDs (Table 3). SNPs *F279Y* and *A257G* were not in linkage disequilibrium ($r^2 = 0.029$, $D' = 0.074$), because linkage of certain alleles was observed only in 7.4% of cases (D'). The significant differences between genotypes by *F279Y* were observed only for daughters' protein yield ($R^2 = 60\%$) and followed $AA > TT > TA$ (Table 7). Considering that data for DMPT were available only for 1 sire with genotype AA the pattern found stay to be dubious. DYD for protein content (%) is close to statistical significance and higher for TT genotype. The genotypes by *A257G* followed $AA > AG > GG$ for milk and protein yield (kg) in daughters, demonstration reversed ($GG > AG > AA$) trend for the same DYDs (Table 8). Both trends can be shifted by uncommon GG genotype.

Table 6. DMPT and DYDs by *L127V* genotype.

Genotype	CC	CG	GG	P	R ²
DMPTs					
Milk yield (kg)	-	7329.17±369.19	9042.67±72.33	0.042	-
Fat yield (kg)	-	291.75±16.99	364.67±3.33	0.057	-
Protein yield (kg)	-	261.30±9.81	320.67±2.33	0.008	-
Fat content (%)	-	3.97±0.13	4.03±0.01	0.823	-
Protein content (%)	-	3.38±0.12	3.54±0.01	0.506	-
BBV (pt)	583.00	632.74±96.35	537.50±73.53	0.905	0.009
DYDs					
Milk yield (kg)	116.00	261.32±79.23	124.50±23.41	0.695	0.034
Fat yield (kg)	571.00	620.16±97.38	308.50±48.32	0.374	0.089
Protein yield (kg)	34.00	31.84±2.70	15.50±4.27	0.001	0.462
Fat content (%)	24.00	22.82±3.60	18.00±0.71	0.808	0.022
Protein content (%)	0.12	0.08±0.03	-0.08±0.03	0.084	0.211
Protein content (%)	0.06	0.05±0.02	0.07±0.02	0.900	0.011

Table 7. DMPT and DYDs by *F279Y* genotype.

Genotype	TT	TA	AA	P	R ²
DMPTs					
Milk yield (kg)	8335.18±614.45	7636.00±391.05	11488.00	0.159	0.217
Fat yield (kg)	330.36±22.21	296.17±19.39	454.00	0.115	0.251
Protein yield (kg)	308.67±9.77	247.83±13.48	360.00	0.002	0.603
Fat content (%)	3.98±0.14	3.87±0.10	3.95	0.854	0.021
Protein content (%)	3.46±0.13	3.25±0.08	3.13	0.392	0.134
BBV (pt)	563.74±75.06	806.00±209.92	895.00±312.00	0.263	0.105
DYDs					
Milk yield (kg)	627.32±479.30	483.17±225.92	231.50±115.50	0.951	0.004
Fat yield (kg)	490.84±76.51	846.17±203.69	697.50±126.50	0.132	0.155
Protein yield (kg)	23.47±3.39	33.50±7.20	39.00±5.00	0.207	0.123
Fat content (%)	19.41±2.53	28.17±7.66	26.00±2.00	0.329	0.096
Protein content (%)	0.06±0.04	-0.01±0.03	0.12±0.01	0.512	0.054
Protein content (%)	0.07±0.02	-0.01±0.03	0.04±0.02	0.051	0.237

Table 8. DMPT and DYDs by *A257G* genotype.

Genotype	AA	AG	GG	P	R ²
DMPTs					
Milk yield (kg)	8852.08±463.06	7213.43±673.32	4717.50±256.50	0.001	0.524
Fat yield (kg)	351.33±14.22	280.86±30.43	176.50±9.50	0.001	0.588
Protein yield (kg)	292.42±11.05	276.80±26.89	-	0.526	-
Fat content (%)	4.01±0.13	3.86±0.06	3.74±0.00	0.363	0.096
Protein content (%)	3.34±0.11	3.44±0.07	-	0.588	-
BBV (pt)	632.00±99.38	752.00±79.28	1027.00±111.00	0.134	0.129
DYDs					
Milk yield (kg)	732.15±453.96	86.00±22.31	50.40±16.40	0.552	0.040
Fat yield (kg)	550.95±86.90	681.29±148.38	1152.60±145.40	0.014	0.256
Protein yield (kg)	28.50±3.18	22.43±7.41	51.60±4.40	0.006	0.296
Fat content (%)	21.40±3.05	24.40±3.96	24.00	0.889	0.010
Protein content (%)	0.07±0.03	-0.03±0.03	0.14±0.01	0.075	0.164
Protein content (%)	0.05±0.02	0.05±0.02	0.06	0.974	0.002

Discussion

CANP316 and *CAST282* were previously found to be associated with meat quality traits: meat tenderness by decreased Warner-Bratzler shear force, overall liking and some carcass traits (Gill et al., 2009, Schenkel et al., 2006) and therefore are predominantly studied in beef cattle breeds (Fedota et al., 2017). For northern Australian beef cattle Collis et al. (2012) revealed association between C-allele of *CANP316* (*CAPN1:c.947C>G*) and decreased ability to ovulate prior to weaning, which may affect

calving interval for cows and fat depth between last two ribs. Their other findings included effect of another SNPs in *CAPN* and *CAST* genes (*CAPN1:g.6545C>T* or *CAPN1-4751* and *CAST:c.2832A>G*) on serum concentration of insulin-like growth factor I (IGF-I, ng/ml). C- alleles for *CAPN1-4751* being associated with meat tenderness led to decreased IGF-I concentration contrary to A-allele of *CAST:c.2832A>G*. IGF-I receptors are present in bovine mammary tissue, whereas serum concentrations of IGF-I in lactating cows increases, as well as close arterial infusion of IGF-I into the mammary gland increases milk yield (Cohick, 1999). Therefore observed effects of SNPs *CANP316* and *CAST282* on DYDs and daughters' milk performance traits in present study could be mediated via IGF-I concentration. Antagonistic genetic correlations of other production traits in steers with age at puberty in heifers was found by Johnston et al. (2009) for two tropical beef genotypes in northern Australia. Cui et al. (2016) discovered the effect of *g.-1256 A > C* in the promoter region of *CAPN1* on semen quality traits in Chinese Holstein bulls, having greater sperm motility in bulls with the genotype CC than in those with the genotype AA.

The observed increase in protein content (%) for CC genotype of *CANP316* can be explained by the higher Ca^{2+} -mediated activity of cytoplasmic calpain involved in the regulation of apoptosis, cell differentiation, synaptic transmission and metabolism of muscle proteins (Miquel et al., 2009). In presence of highly active calpain, the effect of muscle proteins destruction in the smooth muscle of the vessels can lead to the increased low-molecular proteins concentration excreted in milk. The number of C-alleles for SNP *CAST282* is associated with a decrease in calpastatin activity (Schenkel et al., 2006) its effect is mediated through calpain activity regulation, and therefore will be less pronounced. The fat content depends on the metabolism of fatty acids, in particular acetic acid and glycerin, localized in Golgi apparatus. The highest fat content in the milk of daughters of heterozygous bulls for both SNPs can be explained by the intermediate activity of the calpain system. Under low Ca^{2+} concentration, overactive calpain being inhibited by subactive calpastatin (CC genotype for SNP *CAST282*), primarily triggers the destruction of the cytoskeleton, which destabilizes the Golgi apparatus and stops the synthesis of fatty acids.

Considering pivotal role of IGF-I in lactation and association between GH, GHR and serum IGF-I concentration (Ge et al., 2003, Mullen et al., 2011) it would appear reasonable that SNPs studied affect milk traits. Ge et al. used IGF-I as predictor of growth traits during aging and proved the association between SNP *A257G* with mean serum IGF-I concentration.

According Mullen et al. (2010) and Balogh et al. (2008) GH is related with IGF via stimulation its release from the liver and is key in the control of nutrient utilization and partitioning. By *L127V* cows had decreased insulin sensitivity in CG compared to CC cows (Balogh 2008), therefore CG cows had higher 305days lactation yield, than CC cows; GG cows were not presented in population analyzed. Otherwise, our data demonstrate the significant superiority of GG cows over CG for milk, protein and fat yield, higher milk yield (%) for DYDs. In review by Kovacs et al. (2006) there were significant effect of C-allele and CC-genotype on milk traits in general, with higher frequency than G allele or GG genotype. The observed effect on daughters' milk performance traits was supported for Ayrshire, Holstein and Jersey bulls (Sabour et al., 1997) and Canadian HF AI bulls (Sabour & Lin 1996). Therefore we can conclude opposite effect on milk performance traits for daughters of sires studied and lactating cows.

Given the interaction between growth hormone and leptin during the regulation of metabolic processes, the noticed association with milk parameters can be explained as mediated through leptin, which relationship with fat content and other milk characteristics was shown before (Trakovicka et al., 2013).

F279Y and *A287G* are the SNPs in the GHR, therefore they can modulate effects of GH. The effects of *F279Y* reported by different authors are discordant, while Komisarek et al. (2011) reported significant advantages of TT over TA genotype for milk, protein and fat yield for Jersey cows, and Rahmatalla et al. (2011) observed positive association between A-allele and milk, lactose yield and negative association with somatic cell count. Blott et al. (2003) noted the association for A-allele with increased milk yield by 67-112 kg per lactation and showed a strong relationship with protein content, totally characterizing increased protein yield in Holstein-Frisian and Jersey breeds.

The absence of SNP ***A257G*** effect on the analyzed parameters of daughters may be explained by the weak effect of this polymorphic variant with respect to the progeny productivity. We noted that the daughters of bulls with the AA genotype had higher measures compared to the genotype AG, except protein yield. Olenski et al. (2010) found that A-allele had a positive association with milk quality parameters: fat content – by 0.1%, fat yield – by 18.5 kg and protein yield – by 9.1 kg. Waters et al. (2010) estimated allele substitution effect A/G for all milk performance traits as negative and weak but insignificant, although it was significant for growth performance and size. Hradecka et al. (2008) observed the same trend for daughters of German Holstein sires – AA=AG>GG, with fat yield +22.55 kg and fat content -0.058%, whereas milk yield decreased – AA>AG>GG. Thus, discrepancy between observed effects in daughters of bulls and cows for one allele was noted and it was suggested instability of the observed effect determined by polygenic control of these characteristics.

Conclusion

Therefore, high activity of the calpain-calpastatin system enzymes is not associated with the . The fat content and protein yield is higher in daughters of bulls with genotypes *VV* for SNP ***L127V*** and *FF* for SNP ***F279Y***.

Our results confirm the significant association of the SNPs studied and milk performance traits in daughter produced by Ukrainian dairy sires. Furthermore, we provided new association of SNPs *CANP316* and *CAST282* with milk traits, because these SNPs are predominantly known as markers of meat tenderness and quality. We suggest that mechanisms that underlay in high yield are not associated with improvement of productive characteristics in dairy cattle. The results obtained allow to predict the effects of SNP of individual genes on economically important characteristics of the progeny of the estimated bulls, although it is necessary to conduct further studies for of this SNPs effects on reproductive traits in dairy cattle.

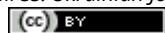
References

- Aliloo, H., Pryce J. E., Gonzalez-Recio, O., Cocks, B. G., Hayes, B. J. (2015). Validation of markers with non-additive effects on milk yield and fertility in Holstein and Jersey cows. *BMC Genetics*, 16 (89). doi: 10.1186/s12863-015-0241-9.
- Amos, W., Driscoll, E., Hoffman, J. I. (2011). Candidate genes versus genome-wide associations: which are better for detecting genetic susceptibility to infectious disease? *Proceedings of the Royal Society B: Biological Sciences*, 278 (1709), 1183–1188. doi: 10.1098/rspb.2010.1920
- Balogh, O., Kovacs, K., Kulcsar, M., Gaspard, A., Zsolnai, A., Katai, L., Pecs, A., Fesus, L., Butler, W. R., Huszenicza, G. (2009). Alu polymorphism of the bovine growth hormone (GH) gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cows. *Theriogenology*, 71 (4), 553–559. doi: 10.1016/j.theriogenology.2008.06.032.
- Berry, D. P., Friggens, N. C., Lucy, M., Roche, J. R. (2016). Milk Production and Fertility in Cattle. *Annual Review of Animal Biosciences*, 4, 269–290. doi: 10.1146/annurev-animal-021815-111406
- Blott, S., Kim, J.-J., Moiso, S., Schmidt-Küntzel, A., Cornet, A., Berzi, P., Cambisano, N., Ford, C., Grisart, B., Johnson, D., Karim, L., Simon, P., Snell, R., Spelman, R., Wong, J., Vilkk, J., Georges, M., Farnir, F., Coppieters, W. (2003). Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics*, 163, 253–266. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1462408/pdf/12586713.pdf>
- Cochran, S. D., Cole, J. B., Null, D. J., Hansen, P. J. (2013). Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. *BMC Genetics*, 14 (49). doi: 10.1186/1471-2156-14-49
- Cohick, W. S. (1998). Role of the insulin-like growth factors and their binding proteins in lactation. *Journal of Dairy Science*, 81 (6):1769–77. doi: 10.3168/jds.S0022-0302(98)75746-7
- Collis, E., Fortes, M. R. S., Zhang, Y., Tier, B., Schutt, K., Barendse, W., Hawken, R. (2012). Genetic variants affecting meat and milk production traits appear to have effects on reproduction traits in cattle. *Animal Genetics*, 43 (4), 442–446. doi:10.1111/j.1365-2052.2011.02272.x
- Cui, X., Sun, Y., Wang, X., Yang C., Ju, Z., Jiang, Q., Zhang, Y., Huang, J., Zhong, J., Yin, M., Wang, C. (2016). A g.-1256 A > C in the promoter region of CAPN1 is associated with semen quality traits in Chinese Holstein bulls. *Reproduction*, 152 (1), 101–109. doi: 10.1530/REP-15-0535
- Ge, W., Davis, M. E., Hines, H. C., Irvin, K. M., Simmen, R. C. (2003). Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *Journal of Animal Science*, 81 (3), 641–648. doi: 10.2527/2003.813641x
- Fedota, O. M., Lysenko, N. G., Ruban, S. Y., Kolisnyk, O. I., Goraychuk, I. V. (2017). The effects of polymorphisms in growth hormone and growth hormone receptor genes on production and reproduction traits in Aberdeen-Angus cattle (*Bos taurus* L., 1758). *Cytology and Genetics*, 51 (5) 352–360. doi: 10.3103/S0095452717050024
- Gill, J. L., Bishop, S. C., McCorquodale, C., Williams, J. L., Wiener, P. (2009). Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*, 41 (36). doi: 10.1186/1297-9686-41-36.
- Goll, D. E., Thompson, V. F., Li, H., Wei, W., Cong, J. (2003). The calpain system. *Physiological Review*, 83, 731–801. doi: 10.1152/physrev.00029.2002
- Hadi, Z., Atashi, H., Dadpasand, M., Derakhshandeh, A., Ghahramani, Seno M. M. (2015). The relationship between growth hormone polymorphism and growth hormone receptor genes with milk yield and reproductive performance in Holstein dairy cows. *Iranian Journal of Veterinary Research*, 16 (3), 244–248. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4782692/pdf/ijvr-16-244.pdf>
- Hill, W. G., Robertson, A. (1968). Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics*, 38(6):226–231. doi: 10.1007/BF01245622.
- Hradecka, E., Citek, J., Panick, L., Rehout, V., Hanusova, L. (2008). The relation of GH1, GHR and DGAT1 polymorphisms with estimated breeding values for milk production traits of German Holstein sires. *Czech Journal of Animal Science*, 53 (6), 238–245. Retrieved from <http://www.agriculturejournals.cz/publicFiles/01545.pdf>
- Hull, K. L., Harvey, S. (2014). Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. *International Journal of Endocrinology*. doi: 10.1155/2014/234014.
- Iso-Touru, T., Tapio, M., Vilkk, J., Kiseleva, T., Ammosov, I., Ivanova, Z., Popov, R., Ozerov, M., Kantanen, J. (2016). Genetic diversity and genomic signatures of selection among cattle breeds from Siberia, eastern and northern Europe. *Animal Genetics*, 47 (6), 647–657. doi: 10.1111/age.12473
- Johnston, D. J., Barwick, S. A., Corbet, N. J., Fordyce, G., Holroyd, R. G., Williams, P. J., Burrow, H. M. (2009). Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. *Animal Production Science*, 49 (5-6), 399–412. doi: 10.1071/EA08276
- Komisarek, J., Michalak, A., Walendowska, A. (2011). The effects of polymorphisms in DGAT1, GH and GHR genes on reproduction and production traits in Jersey cows. *Animal Science Papers and Reports*, 29 (1), pp. 29–36. Retrieved from <http://archiwum.ighz.edu.pl/files/objects/7501/66/strona29-36.pdf>
- Kovacs, K., Volgyi-Csik, J., Zsolnai, A., Gyorkos, I., Fesus, L. (2006). Associations between the Alu polymorphism of growth hormone gene and production and reproduction traits in a Hungarian Holstein-Friesian bull dam population. *Archiv Tierzucht, Dummerstorf*, 49 (3), 236–249. doi: 10.5194/aab-49-236-2006
- Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K. (2013). cluster: Cluster Analysis Basics and Extensions. R package version 1.14.4.

- Miquel, M. C., Villareal, E., Mezzadra, C., Melucci, L., Soria, L., Corva, P., Schor, A. (2009). The association of CAPN1 316 marker genotypes with growth and meat quality traits of steers finished on pasture. *Genetics and Molecular Biology*, 32 (3), 491–496. doi: 10.1590/S1415-47572009000300011
- Mullen, M. P., Lynch, C. O., Waters, S. M., Howard, D. J., O'Boyle, P., Kenny, D. A., Buckley, F., Horan, B., Diskin, M. G. (2011). Single nucleotide polymorphisms in the growth hormone and insulin-like growth factor-1 genes are associated with milk production, body condition score and fertility traits in dairy cows. *Genetics and Molecular Research*, 10 (3), 1819–1830. doi: 10.4238/vol10-3gmr1173.
- Olenski, K., Suchocki, T., Kaminski, S. (2010). Inconsistency of associations between growth hormone receptor gene polymorphism and milk performance traits in Polish Holstein-Friesian cows and bulls. *Animal Science Papers and Reports*, 28 (3), 229–234. Retrieved from http://free-journal.umm.ac.id/files/file/str_229-234.pdf
- Ortega, M. S., Denicol, A. C., Cole, J. B., Null, D. J., Hansen, P. J. (2016). Use of single nucleotide polymorphisms in candidate genes associated with daughter pregnancy rate for prediction of genetic merit for reproduction in Holstein cows. *Animal Genetics*, 47 (3), 288–297. doi: 10.1111/age.12420
- Rahmatalla, S. A., Muller, U., Strucken, E. M., Reissmann, M., Brockmann, G. A. (2011). The F279Y polymorphism of the GHR gene and its relation to milk production and somatic cell score in German Holstein dairy cattle. *Journal of Applied Genetics*, 52 (4), 459–465. doi: 10.1007/s13353-011-0051-3
- Ruban, S., Fedota, O., Lysenko, N., Kolisnik, A., Goraichuk, I., Ponko, L. (2017). Association of single nucleotide polymorphism in the calpain/calpastatin system genes with quantitative traits of Aberdeen-Angus. *The Animal Biology*, 19 (1), 100–110. doi: 10.15407/animbiol19.01.100
- Sabour, M. P., Lin, C. Y., Lee, A. J., McAllister, A. J. (1996). Association between milk protein genetic variants and genetic values of Canadian Holstein bulls for milk yield traits. *Journal of Dairy Science*, 79 (6), 1050–1056. doi: 10.3168/jds.S0022-0302(96)76458-5
- Sabour, M. P., Lin, C. Y., Smith, C. (1997). Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. *Journal of Animal Breeding and Genetics*, 114 (1-6), 435–442. doi: 10.1111/j.1439-0388.1997.tb00529.x.
- Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., Wilton, J. W. (2006). Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, 84, 291–299.
- Suchocki, T., Wojdak-Maksymiec, K., Szyda J. (2016). Using gene networks to identify genes and pathways involved in milk production traits in Polish Holstein dairy cattle. *Czech Journal of Animal Science*, 61 (11), 526–538. doi: 10.17221/43/2015-CJAS
- Szyda, J., Ptak, E., Komisarek, J., Zarnecki, A. (2008). Practical application of daughter yield deviations in dairy cattle breeding. *Journal of Applied Genetics*, 49 (2), 183–191. doi: 10.1007/BF03195611
- Trakovicka, A., Moravcikova, N., Kasarda, R. (2013). Genetic polymorphisms of leptin and leptin receptor genes in relation with production and reproduction traits in cattle. *Acta Biochimica Polonica*, 60 (4), 783–787. Retrieved from http://www.actabp.pl/pdf/4_2013/783.pdf
- Viitala S., Szyda J., Blott S., Schulman N., Lidauer M., Maki-Tanila A., Georges M., Vilkki J. (2006). The role of the bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in Finnish Ayrshire dairy cattle. *Genetics*, 173 (4), 2151–2164. doi: 10.1534/genetics.105.046730
- Waters, S. M., McCabe, M. S., Howard, D. J., Giblin, L., Magee, D. A., MacHugh, D. E., Berry, D. P. (2011). Associations between newly discovered polymorphisms in the Bos taurus growth hormone receptor gene and performance traits in Holstein–Friesian dairy cattle. *Animal Genetics*, 42 (1), 39–49. doi: 10.1111/j.1365-2052.2010.02087.x.

Citation:

Fedota, O., Lysenko, N., Mitiohlo, L., Ruban, S. (2018). Effects of 5 SNPs on daughters' milk performance traits produced by Ukrainian dairy sires. *Ukrainian Journal of Ecology*, 8(1), 939–947.



This work is licensed under a Creative Commons Attribution 4.0. License