AGRICULTURAL BIOLOGY,
2018, V. 53, № 4, pp. 723-734
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.4:636.082:575.1

doi: 10.15389/agrobiology.2018.4.723eng
doi: 10.15389/agrobiology.2018.4.723rus

EFFECTS OF GENOTYPES FOR *IGF2*, *CCKAR* AND *MC4R* ON THE PHENOTYPIC ESTIMATIONS AND BREEDING VALUES FOR PRODUCTIVE TRAITS IN PIGS

E.E. MELNIKOVA[1], N.V. BARDUKOV[1], M.S. FORNARA[1], O.V. KOSTYUNINA[1], A.A. SERMYAGIN[1], A.M. ZAITSEV[2], N.A. ZINOVIEVA[1]

[1]*Ernst Federal Science Center for Animal Husbandry,* Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail melnikovaee@vij.ru, bardukv-nikolajj@mail.ru, margaret-fornara@gmail.ru, kostolan@yandex.ru, alex_sermyagin85@mail.ru, n_zinovieva@mail.ru (✉ corresponding author); [2]*All-Russian Research Institute for Horse Breeding,* Federal Agency of Scientific Organizations, pos. Divovo, Rybnovskii Region, Ryazan Province, 391105 Russia, e-mail amzaitceff@mail.ru
ORCID:
Melnikova E.E. orcid.org/0000-0002-7498-1871
Bardukov N.V. orcid.org/0000-0002-5497-2409
Fornara M.S. orcid.org/0000-0002-8844-177X
Kostyunina O.V. orcid.org/0000-0001-8206-3221
Sermyagin A.A. orcid.org/0000-0002-1799-6014
Zaitsev A.M. orcid.org/0000-0003-4260-602X
Zinovieva N.A. orcid.org/0000-0003-4017-6863
The authors declare no conflict of interests
Acknowledgements:
The equipment of the Sharing Center for Farm Animal Bioresources and Bioengineering (FSC for Animal Husbandry) was used.
Supported financially by Ministry of Education and Science of the Russian Federation (the project unique identifier RFMEFI60417X0182)
Received March 21, 2018

Abstract

Development of programs for marker-assisted selection has to be based on genetic polymorphisms, whose effect on the production traits and breeding values of animals is reliable and significant. Prospects for the use of genomic selection in pigs are associated with the development of low-density (LD) DNA arrays, which include the SNPs (single nucleotide polymorphisms) selected by the results of genome-wide association studies (GWAS) with HD-panels. Genes of insulin-like growth factor 2 (*IGF2*), cholecystokinin receptor A (*CCKAR*) and melanocortin 4 receptor (*MC4R*) are of interest for inclusion in LD-panels. Numerous studies have shown a significant effect of these genes on feed conversion rate, growth rate, meat content, and fat deposition. The aim of this work was to evaluate the effect of complex genotypes for *IGF2*, *CCKAR* and *MC4R* on growth and carcass traits of the Landrace and Large White pigs raced in Russia. In total, 1262 animals, including Large White (n = 667) and Landrace pigs (n = 595) were studied. Pig phenotypes were determined for muscle depth (MD, mm), adjusted age at 100 kg (AGE100, day) and back fat thickness (BF, back fat) at three points: BF1 (at ribs 6-7, mm), BF2 (at rib 10, mm), BF3 (at 14 rib, mm). DNA was extracted from tissue samples (ear pluck) using a DNA-Extran-2 Kit (Sintol, Russia). Polymorphism of *IGF2* was determined by real-time PCR. Causative SNPs in *CCKAR* and *MC4R* genes were defined by multiplex PCR with FLASH detection. The allele frequencies of DNA-markers were pA = 27.2 % and pA = 86.3 % for *IGF2*, pA = 0.6 % and pA = 21.1 % for *CCKAR*, pA = 54.1 % and 60.0 % for *MC4R* in Landrace and Large White pigs, respectively. The heritability coefficients (h^2) were 0.204-0.242 for BF1, BF2, and BF3, 0.309 for MD, and 0.366 for AGE100. We developed an equation model for the pig's breeding traits and found the significant effects of fixed factors in the model (breed, sex, year of birth), including specific genotypes for the analyzed genes on the phenotypic variations (for *IGF2* and *MC4R* on BF1, BF2 and BF3, $P > 0.95$), and estimated breeding values (EBV) for growth and carcass traits (for each of the three markers the ratio of additive genetic variation ranged from 0.5 to 7.6 %, $P > 0.95$-0.999). We identified the economically desired alleles for *IGF2* (allele *A*) and *MC4R* (allele *A*) genes. Animals which carried the homozygous genotypes for the desired alleles (*AA* for both of *IGF2* and *MC4R* genes) were characterized by the significantly better scores for analyzed traits, estimated by least squares method, comparing to the individuals which were homozygous for the alternative allele *G*. The additive compensating effect of genotypes' combinations for *IGF2* and *MC4R* on the pig growth traits was established. The animals with the highest number of the *A* alleles for *IGF2* and *MC4R* had preferable characteristics for the back fat thickness comparing to animals with *GG* genotypes (for both DNA markers). The differences between groups of animals carrying in their genotypes from four to single copy of the

A alleles comparing to animals which do not have *A* alleles (*GG* genotypes for both markers) varied from 7.9 to 21.0 % for BF1, from 8.5 to 21.4 % for BF2, from 9.9 to 22.6 % for BF3, and from 2.8 to 3.2 % for MD. In this regard, the *IGF2* and *MC4R* genotypes can be used in breeding programs of Large White and Landrace pigs raced in Russia to select the pigs with desired growth and carcass characteristics.

Keywords: pigs, Large White breed, Landrace, *IGF2*, *CCKAR*, *MC4R* genes, polymorphisms, estimated breeding value (EBV), growth and carcass traits

The genomic selection is thought of as a promising strategy of genetic improvement of livestock, including pigs [1-3]. The integration of genome methods in pig breeding became possible due to the development of commercial SNP-arrays (single nucleotide polymorphisms) for highly productive genotyping with density varying from 10.2 thousand to more than 650 thousand SNPs [4]. If in livestock breeding the genomic selection programs are implemented on the basis of using SNP-arrays of medium density (MD) and high density (HD) [2, 5, 6], in pig breeding the application of MD- and HD-arrays is economically unjustified. The implementation of genomic selection of pigs is associated with the development of low density DNA-matrixes with inclusion of SNPs in them selected by the results of GWAS (genome-wide association studies) analysis using MD- and HD-arrays [7-10]. In order to increase the information content of LD-arrays, they additionally include SNPs localized in "primary" genes associated with QTL. Presently, more than ten genes are known that have a noticeable effect on economic traits of pigs [11]. Their inclusion in LD-arrays will facilitate the improved forecast accuracy of estimated breeding values (EBV). In order to select economically significant SNPs, their impact on economic traits in populations should first be evaluated, which implements the programs of marker and genomic selection. The insulin-like growth factor 2 (*IGF2*) genes, Cholecystokinin A receptor (*CCKAR*) and Melanocortin 4 receptor (*MC4R*) act as potential DNA markers for inclusion in LD-arrays.

The *IGF2* gene is localized to the distal end of SSC2 pig chromosome [12]. G3072A has been identified, which has a significant impact on the rate of growth, muscle gain and fat deposition of these animals [13-15]. Subsequently, the effect of this mutation was confirmed in numerous studies conducted in various pig populations of both foreign (16, 17) and domestic selection [18, 19]. The advantage of using *IGF2* as a DNA marker is attributable to its paternal nature, i.e. the effect of paternal genotype for *IGF2* manifests itself in progeny regardless of maternal genotype [13]. Another advantage of *IGF2* is related to the positive impact of potentially "desirable" allele A on reproductive qualities from the point from view of feeding qualities [18].

CCKAR is one of the principal receptors participating in hunger regulation [20, 21]. The *CCKAR* gene of pigs is mapped on SSC8 chromosome [22]. An economically significant SNP A179G (exon 1) [23] associated with the growing power and feed conversion rate of pigs is known: the animals with at least *G* allele are on average characterized by at least a 3% higher daily body weight gains and at least 5 % higher cost of feed per 1 kg of weight gain [23, 24].

MC4R is involved in regulation of hypothalamic-pituitary-adrenal axis (HPA) via vasopressin and corticotropic neurons [25]. Due to MC4R expression in the brain region regulating appetite its connection with feed consumption and energy balance can safely be presumed. The *MC4R* locus is mapped on SSC1 chromosome in the q22-27 region [26]. A G→A mutation is known, which results in Asp298Asn ammo-acid replacement, which establishes a connection to the increase of fatness, body weight gain and increased feed consumption [26]. The population-genetic studies showed significant differences in *MC4R* (p_A = 0.24-1.00) allele frequencies both among and inside varying pig breeds [27-29]. A

connection has been identified between allocations of *MC4R* alleles with the selection strategies deployed. For instance, lines of Large White pigs selected for high fleshing and low feed conversion rate, the *MC4R A* allele frequency was $p_A = 0.52$ against $p_A = 1.00$ in the line selected for low fleshing and high feed conversion rate [30]. The available data about the effect of *MC4R* polymorphism on productive indicators are not universal. However, a marked impact of this gene on daily live weight gain of purebred pigs [28-30] and crossbred pigs [31-33], feed consumption [27], as well as muscle weight gain and carcass fat content [33-35] has been identified. A significant effect of *MC4R* polymorphism has been identified, which is manifested with regard to feed consumption which, in turn, influenced back fat and growth rate (variations of 5-8%) [27]. The impact of this mutation on the aforementioned characters was noted in almost all analyzed commercial lines. The maximum mean differences for combined genotypes constituted 2 mm for back fat, 70 g/day for average daily live weight gain and 2 % for meat content. K. Salajpal at al. [36] determined the impact of *MC4R* genotype on back fat (*GG* < *AA*) and percentage content of meat in the carcass (*GG* > *AA*, $p < 0.05$) in marketable pigs.

The inclusion of several DNA markers in selection programs requires evaluation of their combined effect on manifestation of economic traits and establishing quantitative relations with the estimated breeding values (EBV).

In this study, for the first time we analyzed the associations of polymorphisms of several genes involved in regulating a number of metabolic and physiological processes with carcass and growth traits of pigs bred in Russia, and identified a reliable connection between certain *IGF2*, *CCKAR* and *MC4R* genotypes with variability of these attributes.

Our goal was to study the combined effect of *IGF2*, *CCKAR* and *MC4R* genotypes on carcass and growth traits of Large White and Landrace pigs.

Techniques. In 2017-2018, 1262 pigs (*Sus scrofa*) were selected to form a test sample which included Large White pigs ($n = 667$) and Landrace pigs ($n = 595$) of Russian selection (Selection and Hybrid Center LLC, Voronezh region). The pig phenotypes were identified using muscle depth (MD), mm, age of 100 kg live weight (AGE_{100}, days) and back fat (BF) in three points: BF1 (in the area of 6-7 rib, mm), BF2 (in the area of 10 rib, mm) and BF3 (in the area of 14 rib, mm).

The DNA was separated from boar tissue samples (ear notch) using a set of DNA-Extran-2 (Syntol R&D company, Russia) reagents. The DNA concentration and quality were evaluated with a Qubit 2.0 desktop fluorimeter (Invitrogen/Life Technologies, USA) and NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, USA). *IGF2*gene polymorphism (G→A in position 16144, Accession No. AY242112) was determined via PCR method in real time using a QuantStudio 5 device (Thermo Fisher Scientific, USA). The single nucleotide polymorphisms in *CCKAR* gene (A→G in position +179, Accession No. DQ496228.1) and *MC4R* gene (G→A in position +1426, Accession No. NM_214173.1) were defined by multiplex PCR method with FLASH-detection (fluorescent amplification-based specific hybridization) at the end point using high performance genotyping system Fluidigm EP1 (Fluidigm Corporation, USA).

The development of a model to evaluate the degree of impact of *IGF2*, *CCKAR*, *MC4R* animal genotypes for variability of test parameters included consideration of a number of equations, which, apart from the studied genetic factors, contained the factors reflecting paratypic effects, possibly affecting the phenotypic manifestations of carcass and growth traits. Using the multivariate analysis of variance (MANOVA) model equations with a varying number of fixed factors were tested. The equation characterized with the lowest error variance value was selected for

further analysis, which was used to calculate mean appraisal values using the least squares method (LSM) via STATISTICA 10 (StatSoft, Inc., USA) software:

$$y = \mu + B_i + Sex_j + Year_k + G_l + e,$$

where y is the calculated indicators of carcass and growth traits (BF1, BF2, BF3, MD, AGE_{100}); μ is mean population constant; B_i is "breed" factor (Landrace, Large White); $Year_k$ is the year of birth of an animal (years 2009-2017); G_l is the effect of the genotype for each of *IGF2*, *CCKAR* and *MC4R* markers; e is a random error (unallocated variance).

The estimated breeding values of livestock units when comparing with marker genotypes were calculated using a similar model using additive kinship matrix information according to the BLUP Animal Model (n = 1752, including 1262 animals with genotypes of corresponding genes) method. The EBV calculations and analysis of variation components were conducted using BLUPF90 (37) program family.

When studying the combined impact of genotype combination for two markers (*IGF2*/*MC4R*), all animals were divided into groups by desirable allele *A* frequency. Group 4*A* (100%) includes pigs with genotype *AA*/*AA* for *IGF2* and *MC4R*; 3*A* pigs (75%) are with *AA*/*AG*, *AG*/*AA*; 2*A* pigs (50%) are with *AA*/*GG*, *AG*/*AG*, *GG*/*AA*; 1*A* pigs (25%) with *AG*/*GG*, *GG*/*AG*; 0A (0 %) means *GG*/*GG*.

Fisher criterion was used to evaluate statistical significance of factors taken into account (*F*-criterion, the ratio between the considered factor variance and residual variance $F = \sigma_f^2/\sigma_e^2$) for the corresponding number of degrees of freedom (df). The accuracy of mean bias of traits for the compared genotype groups was determined using *t*-Student criterion for the corresponding number of degrees of freedom and levels of confidence factors $P > 0.95$, $P > 0.99$, $P > 0.999$.

Results. The studied pig sample had relatively high mean values of carcass and growth traits (Table 1). The moderate nature of heritability for studied traits was identified (h^2 = 0.204-0.366), which is in line with the results of works by other authors [38].

1. Characterization of carcass and growth traits in the analyzed sample of Large White and Landrace pigs (*Sus scrofa*) (Selection and Hybrid Center LLC, Voronezh region, 2017-2018)

Traits	*M*	σ	BF1	BF2	BF3	MD	AGE_{100}
BF1	14.74	3.44	0.242[c]	0.931	0.926	0.400	0.070
BF2	12.10	2.92	0.878	0.236[c]	0.969	0.165	0.008
BF3	11.74	2.75	0.807	0.843	0.204[c]	0.216	–0.136
MD	56.36	6.06	0.327	0.325	0.351	0.309[c]	–0.070
AGE_{100}	159.6	8.26	0.116	0.127	0.099	0.099	0.366[c]

N o t e. BF1 — back fat thickness in the region of rib 6-7, mm; BF2 — back fat thickness in the region of rib 10, mm; BF3 — back fat thickness in the region of rib 14, mm; MD — muscle depth, mm; AGE100 — age of 100 kg body weight, days; *M* — mean value of an indicator, σ — mean square deviation; c — diagonally located heritability coefficients h^2 (under the diagonal line are the phenotypic correlations, above the diagonal line are genetic correlations).

In the course of the analysis we identified high positive interrelations among phenotypic (r_1 from +0.807 to +0.878) and genetic (r_2 from +0.926 to +0.969) indicators of BF1-BF2, BF1-BF3, BF2-BF3 trait pairs, which is indicative of uniformity of fat deposit (fatty tissue) of pigs by analyzed control points when they achieve body weight of 100 kg and is considered a positive trait of raised livestock population. At the same time, the values of back fat thickness indicated an average and low (for genetic properties of units) degree of connection with muscle depth and age of live weight gain of 100 kg, i.e., from the genetic point of view they turned out almost completely independent. That being said it seems feasible to conduct genetic evaluation of livestock population for each of the mentioned traits individually, and to include them in integrated evaluation of car-

cass and fattening traits of pigs.

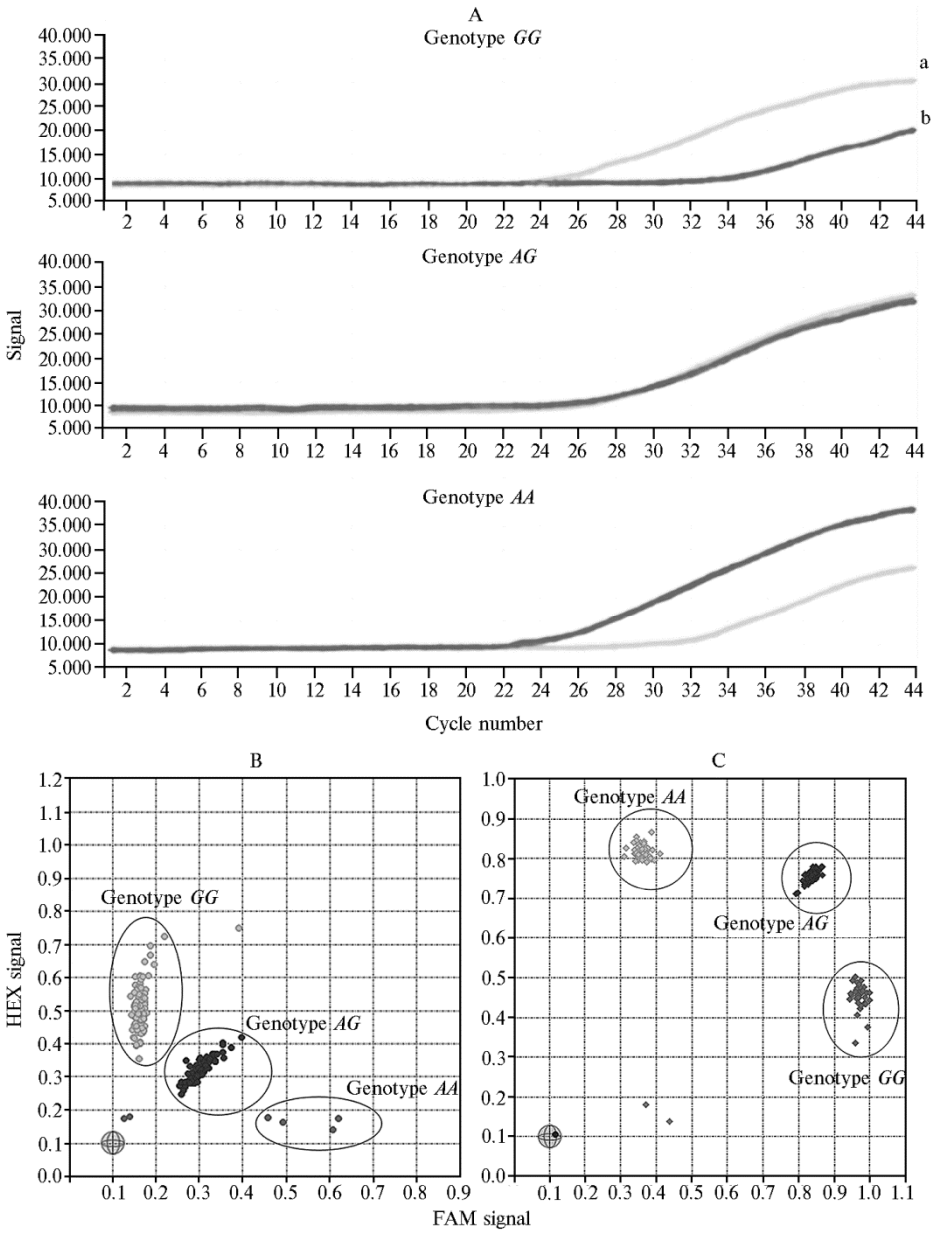

Fig. 1. Genotyping *IGF2*, *CCKAR* and *MC4R* DNA markers of Large White and Landrace pigs (*Sus scrofa*): A — genotyping *IGF2* DNA marker using the allele-specific real time PCR method (QuantStudio 5, Thermo Fisher Scientific, USA), B and C — genotyping *CCKAR* and *MC4R* DNA markers respectively using FLASH-PCR (fluorescent amplification-based specific hybridization) technology with end point detection (Fluidigm EP1, Fluidigm Corporation, USA); a — G allele, b — A allele (Selection and Hybrid Center LLC, Voronezh region, 2017-2018).

The genotyping of *IGF2*, *CCKAR* and *MC4R* using high performance PCR-analysis methods allowed us clearly identify genotypes of DNA markers in question (Fig. 1).

All DNA markers of analyzed pig breeds turned out polymorphous (Fig. 2). The analysis shows lack of essential differences in frequencies of occurrence of *MC4R* genotypes and alleles between Large White and Landrace pig breeds (p_A corresponds to 54.1 and 60.0%). At the same time, uneven distribution of

genotypes and alleles of other DNA markers are identified, i.e. there are p_A 27.2 and 86.3% for *IGF2*, and p_A 0.6 and 21.1% for *CCKAR* for Landrace and Large White pigs respectively.

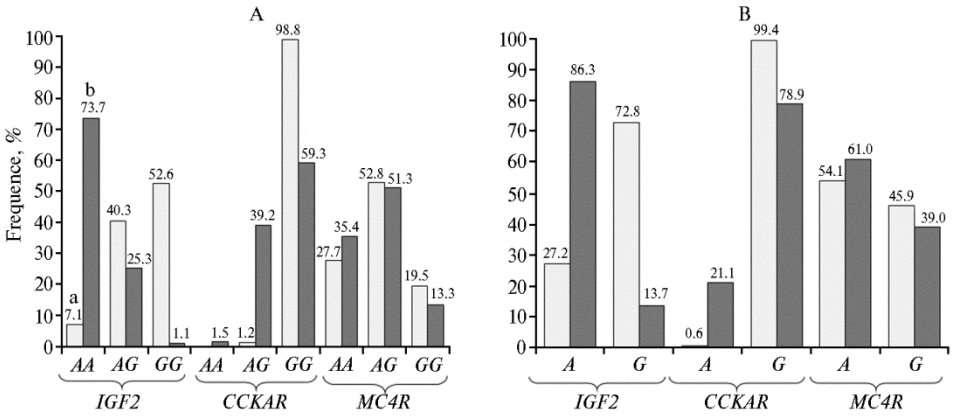

Fig. 2. Distribution of genotype frequencies (A) **and alleles** (B) **of *IGF2*, *CCKAR* and *MC4R* DNA markers in Large White** (a) **and Landrace** (b) **pigs (*Sus scrofa*)** (Selection and hybrid center LLC, Voronezh region, 2017-2018)**.**

2. The reliability values of model factor impact on variance of carcass and growth traits of Large White and Landrace pigs (*Sus scrofa*) based on multivariate analysis of variance without factor interaction (Selection and hybrid center LLC, Voronezh region, 2017-2018)

Factor	df	*F*-criterion				
		BF1	BF2	BF3	MD	AGE_{100}
Breed	2	2.03	3.18*	2.20	0.02	6.01*
Gender	1	12.10*	14.37*	16.87*	0.41	14.50*
Year of birth	8	21.16*	26.85*	17.54*	40.35*	19.35*
IGF2 genotype	2	12.06*	11.67*	13.83*	0.64	2.16
CCKAR genotype	2	0.02	0.24	0.15	0.28	0.06
MC4R genotype	2	21.27*	18.59*	15.66*	2.07	2.81

N o t e. df — number of degrees of freedom; *F*-criterion — statistical Fisher distribution criterion; BF1 — back fat thickness in the region of rib 6-7, mm; BF2 — back fat thickness in the region of rib 10, mm; BF3 — back fat thickness in the region of rib 14, mm; MD — muscle depth, mm; AGE100 — age of 100 kg body weight, days.
* Impact of the factor on variance of the indicator is statistically significant at P > 0.95.

3. The contingency of estimated breeding values (EBV) **for carcass and growth traits of Large White and Landrace pigs (*Sus scrofa*) depending on genotype for *IGF2*, *CCKAR* and *MC4R* genes** (Selection and Hybrid Center LLC, Voronezh region, 2017-2018)

Marker	Comparable genotype pair	EBV				
		BF1	BF2	BF3	MD	AGE_{100}
IGF2	*AA*	–0.086	–0.005	–0.024	–0.519	+0.043
	AG	+0.122	+0.137	+0.122	–0.303	–0.114
	GG	+0.491	+0.450	+0.399	–0.186	–0.042
	F-criterion	33.0^{***}	31.3^{***}	33.6^{***}	3.2^{*}	0.3
	R^2, %	4.99	4.75	5.07	0.51	0.05
CCKAR	*AA*	–0.245	–0.010	–0.038	–2.276	+0.553
	AG	–0.029	+0.059	+0.035	–0.732	+0.155
	GG	+0.172	+0.183	+0.157	–0.247	–0.090
	F- criterion	4.6^{**}	2.5	3.0*	11.4^{***}	0.9
	R^2, %	0.73	0.40	0.48	1.78	0.14
MC4R	*AA*	–0.244	–0.123	–0.132	–0.616	+0.327
	AG	+0.191	+0.207	+0.178	–0.345	–0.120
	GG	+0.599	+0.527	+0.480	+0.073	–0.481
	F- criterion	49.0^{***}	46.4^{***}	51.6^{***}	8.5^{***}	5.6^{**}
	R^2, %	7.24	6.88	7.59	1.33	0.88

N o t e. BF1 — back fat thickness in the region of rib 6-7, mm; BF2 — back fat thickness in the region of rib 10, mm; BF3 — back fat thickness in the region of rib 14, mm; MD — muscle depth, mm; AGE100 — age of 100 kg body weight, days.
*, **, *** Impact of the factor on variance of the indicator is statistically significant at P > 0.95, P> 0.99 and P > 0.999.

In the course of the multivariate analysis of variance (MANOVA) it was observed that for the analyzed group of animals the following factors had significant impact on the variance of productivity: (for BF2 and AGE_{100} traits $P > 0.95$), gender and year of birth (for all traits $P > 0.95$, with the exception of MD), and *IGF2* and *MC4R* market genotypes (for traits BF, $P > 0.95$) (Table 2). A genotype of pigs regarding *CCKAR* marker had no reliable impact on manifestation of productive traits in question.

Of particular interest for the usage of DNA markers in the selection is the study of their impact on estimated breeding values of units (Table 3). Determining the criterion of significance (*F*-criterion) for "genotype for DNA marker" trait enabled identifying reliable impact of each of the genetic traits in question on the change of estimated breeding values for all five traits for *MC4R*, for four out of five traits for *IGF2* (apart from AGE_{100}) and for three traits for *CCKAR* (apart from BF2 and AGE_{100}). The findings confirm the reliable breeding value superiority ($P > 0.95$-0.999) of pigs with *IGF2 AA* genotype over those with *AG* and *GG* genotype in terms of back fat thickness. Furthermore, *AA* pigs stood out by decreased EBV values for MD and AGE_{100}. Identical tendencies are identified for *CCKAR* and *MC4R* markers, for which desirable *AA* genotypes were defined for BF1, BF2 and BF3 genetic values and *GG* genotypes for MD and AGE_{100} traits. The highest value of interconnection linearity between the traits analyzed were observed for trait pairs "genotype for *MC4R* marker—back fat thickness in three points" ($R^2 = 6.88$-7.59 %) (see Table 3).

The identified regularities are confirmed upon comparison of the results received for both pig breeds (Table 4). Furthermore, for Large White pigs statistically significant variances have been determined between *MC4R* homozygous genotype for AGE_{100} trait ($P > 0.95$-0.999), which is indirectly indicative of a relation between this trait and carcass trait (back fat thickness in three measurement points, BF1, BF2 and BF3).

4. The weighted appraisal values for productive traits for *IGF2*, *CCKAR* and *MC4R* genotypes in pigs (*Sus scrofa*) of different breeds obtained by the least squares method (Selection and Hybrid Center LLC, Voronezh region, 2017-2018)

DNA marker	Genotype (*n*)	LSM estimates for productive traits				
		BF1	BF2	BF3	MD	AGE_{100}
		Landrace				
IGF2	*AA* (*n* = 42)	13.69±0.60[a]	10.63±0.48[a]	9.66±0.46[a]	55.18±0.99	160.12±1.40
	AG (*n* = 240)	14.46±0.38	11.67±0.30	10.62±0.29	53.74±0.62	159.84±0.88
	GG (*n* = 313)	15.22±0.39	12.34±0.31	11.20±0.30	53.12±0.65	161.19±0.91
CCKAR	*AA* (*n* = 0)					
	AG (*n* = 7)	14.38±1.32	11.60±1.06	10.29±1.01	54.37±2.17	160.15±3.07
	GG (*n* = 588)	14.68±0.36	11.83±0.28	10.76±0.27	53.63±0.58	160.39±0.83
MC4R	*AA* (*n* = 165)	13.88±0.41[b]	11.11±0.32[b]	10.26±0.31[b]	53.39±0.68	160.44±0.97
	AG (*n* = 314)	14.82±0.37	11.97±0.29	10.80±0.28	53.62±0.62	160.36±0.87
	GG (*n* = 116)	15.84±0.45	12.82±0.36	11.63±0.35	54.19±0.76	160.42±1.07
		Large White				
IGF2	*AA* (*n* = 481)	13.68±0.38[a]	11.25±0.33[a]	10.69±0.32[a]	52.52±0.65	153.71±0.94
	AG (*n* = 165)	14.45±0.44	11.68±0.38	11.22±0.36	52.47±0.75	154.54±1.08
	GG (*n* = 7)	16.64±1.22	13.47±1.07	13.39±1.02	57.20±2.11	158.63±3.03
CCKAR	*AA* (*n* = 10)	13.83±1.05	11.54±0.91	10.87±0.88	51.35±1.80	154.58±2.58
	AG (*n* = 256)	13.85±0.41	11.27±0.36	10.77±0.35	52.45±0.71	153.82±1.02
	GG (*n* = 387)	13.86±0.39	11.39±0.34	10.85±0.32	52.62±0.67	153.96±0.96
MC4R	*AA* (*n* = 231)	13.12±0.42[b]	10.88±0.37[b]	10.37±0.35[b]	51.94±0.73	155.24±1.04[b]
	AG (*n* = 335)	13.81±0.39	11.30±0.34	10.78±0.33	52.65±0.68	153.96±0.98
	GG (*n* = 87)	14.73±0.46	11.97±0.40	11.39±0.39	52.99±0.80	152.43±1.14

Note. BF1 — back fat thickness in the region of rib 6-7, mm; BF2 — back fat thickness in the region of rib 10, mm; BF3 — back fat thickness in the region of rib 14, mm; MD — muscle depth, mm; AGE100 — age of 100 kg body weight, days. [a] The differences with the *GG* (*IGF2*) are statistically significant at $P > 0.95$-0.999. [b] The differences with the *GG* (*MC4R*) are statistically significant at $P > 0.95$-0.999.

IGF2 and *MC4R* DNA markers can not only have a significant and similar impact on carcass and growth traits, but also be interconnected among each

other and have compensatory effect (the additive effect of genes). In order to test this hypothesis we analyzed evaluations of carcass and growth traits of animals depending on genotype combinations for two markers (*IGF2/MC4R*). The findings (Table 5) confirm the validity of the proposed hypothesis relative to the traits of fattening productivity of pigs (back fat thickness in three measurement points). Moreover, the pigs with the best traits are the ones with the highest frequency of occurrence of desirable *A* alleles according to *IGF2* and *MC4R*. The identified regularities confirm the availability of correlative interconnections of *IGF2* and *MC4R* genotypes with carcass and growth traits, which can be used in practical selection.

5. The weighted appraisal values for productive traits for genotype combinations for *IGF2/MC4R* DNA markers in large white and landrace pig groups (*Sus scrofa*) obtained by the least squares method (LSM) (Selection and Hybrid Center LLC, Voronezh region, 2017-2018)

Group	*n*	LSM estimates for productive traits				
		BF1	BF2	BF3	MD	AGE_{100}
4*A*	195	12.96 ± 0.41^{abc}	10.35 ± 0.34^{abc}	9.61 ± 0.33^{abc}	52.71 ± 0.71	160.81 ± 1.02
3*A*	400	13.65 ± 0.35^{ab}	10.85 ± 0.30^{ab}	10.17 ± 0.28^{ab}	53.54 ± 0.61	159.93 ± 0.87
2*A*	339	14.19 ± 0.38^{abeд}	11.25 ± 0.32^{abe}	10.60 ± 0.31^{abe}	53.01 ± 0.66	159.69 ± 0.94
1*A*	258	15.14 ± 0.40^{acde}	12.05 ± 0.34^{acde}	11.18 ± 0.32^{acde}	52.96 ± 0.70	160.21 ± 0.99
0*A*	64	16.43 ± 0.53^{bcde}	13.17 ± 0.45^{bcde}	12.41 ± 0.43^{bcde}	54.47 ± 0.92	160.52 ± 1.32

N o t e. BF1 — back fat thickness in the region of rib 6-7, mm; BF2 — back fat thickness in the region of rib 10, mm; BF3 — back fat thickness in the region of rib 14, mm; MD — muscle depth, mm; AGE100 — age of 100 kg body weight, days..

[a] The differences are statistically significant when comparing with the 0*A* group, P > 0.95-0.999.
[b] The differences are statistically significant when comparing with the 1*A* group, P > 0.95-0.999.
[c] The differences are statistically significant when comparing with the 2*A* group, P > 0.95-0.999.
[d] The differences are statistically significant when comparing with the 3*A* group, P > 0.95-0.999.
[e] The differences are statistically significant when comparing with the 4*A* group, P > 0.95-0.999.

Therefore, proven impact of certain *IGF2*, *CCKAR* and *MC4R* genotypes on variability of carcass and growth traits of Large White and Landrace pigs has been determined. The presence of a desirable genotype for these genes can be used as a factor in marker selection of pigs based on back fat thickness traits (in three measurement points) and muscle depth for *IGF2* (P > 0.95-0.999), and for back fat thickness traits (in three measurement points), muscle depth and age of live weight gain of 100 kg for *MC4R* (P > 0.99-0.999). Connectivity of *CCKAR* gene genotype with genetic estimates for BF1 (P > 0.99) and MD (P > 0.999) traits has been identified. Our findings identify the additive interconnection of genotypes for *IGF2* and *MC4R* markers with fattening pig traits in question and prove superiority of pigs with the highest frequency of a desirable allele above those homozygous for an alternative genotype.

To conclude, presence of a certain genotype for markers in question can conventionally characterize the genetic potential of a pig for its carcass and growth traits. When evaluating and allocating animals to primary selection groups it is expedient to take into consideration both the presence of desirable individual alleles for *IGF2* and *MC4R* markers and combinations of genes displaying proven correlated impact on variance of economically significant traits. When developing DNA biochips, the analyzed mutations associated with the quantitative traits of pig productivity, as reliably significant for breeding value of animal, should be taken into account in the array design. This will improve the efficiency of selection during pig breeding.

REFERENCES

1. Sermyagin A.A., Naryshkina E.N., Karpushkina T.V., Strekozov N.I., Zinov'eva N.A. *Molochnoe i myasnoe skotovodstvo*, 2015, 7: 2-5 (in Russ.).
2. Sermyagin A.A., Gladyr' E.A., Kharitonov S.N., Ermilov A.N., Strekozov N.I., Brem G., Zi-

nov'eva N.A. Genome-wide association study for milk production and reproduction traits in Russian Holstein cattle population. *Agricultural Biology* [*Sel'skokhozyaistvennaya Biologiya*], 2016, 51(2): 182-193 (doi: 10.15389/agrobiology.2016.2.182eng).
3. Zinov'eva N.A., Sermyagin A.A., Kostyunina O.V. *Zhivotnovodstvo Rossii*, 2018, 7: 53-55 (in Russ.).
4. Ramos A.M., Crooijmas R.P., Affara N.A., Amaral A.J., Archibald A.L., Beever J.E., Benedixen C., Churcher C., Clark R., Dehais P., Hansen M. S., Hedegaard J., Hu Z.L., Kerstens H.H., Law A.S., Megens H.-J., Milan D., Nonneman D.J., Rohrer G.A., Rothschild M.F., Smith T.P.L., Schnabel R.D., Van Tassell C.P., Taylor J.F., Wiedmann R.T., Schook L.B., Groenen M.A.M. Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. *PLoS ONE*, 2009, 4(8): 6524 (doi: 10.1371/journal.pone.0006524).
5. Meuwissen T.H.E., Hayes B.J., Goddard M.E. Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 2001, 157(4): 1819-1829.
6. VanRaden P.M., Null D.J., Sargolzaei M., Wiggans G.R., Tooker M.E., Cole J.B., Sonstegard T.S., Faust M.A., Doak G.A. Genomic imputation and evaluation using high-density Holstein genotypes. *J. Dairy Sci.*, 2013, 96(1): 668-678 (doi: 10.3168/jds.2012-5702).
7. Habier D., Fernando R.L., Dekkers JCM. Genomic selection using low-density marker panels. *Genetics*, 2009, 182(1): 343-353 (doi: 10.1534/genetics.108.100289).
8. Huang Y., Hickey J.M., Cleveland M.A., Maltecca C. Assessment of alternative genotyping strategies to maximize imputation accuracy at minimal cost. *Genet. Sel. Evol.*, 2012, 44(1): 25 (doi: 10.1186/1297-9686-44-25).
9. Cleveland M.A., Hickey J.M. Practical implementation of cost-effective genomic selection in commercial pig breeding using imputation. *J. Anim. Sci.*, 2013, 91(8): 3583-3592 (doi: 10.2527/jas.2013-6270).
10. Abell C.E., Dekkers J.C.M., Rothschild M.F., Mabry J.W., Stalder K.J. Total cost estimation for implementing genome-enabled selection in a multi-level swine production system. *Genet. Sel. Evol.*, 2014, 46(1): 32 (doi: 10.1186/1297-9686-46-32).
11. *Pig QTLdb*. Available https://www.animalgenome.org/cgi-bin/QTLdb/SS/index. Accessed May 10, 2018.
12. Van Laere A.-S. From QTL to QTN. *Identification of a quantitative trait nucleotide influencing muscle development and fat deposition in pig. Doctoral thesis.* Swedish University of Agricultural Sciences, Uppsala, 2005.
13. Van Laere A.S., Nguyen M., Braunschweig M., Nezer C., Collette C., Moreau L., Archibald A.L., Haley C.S., Buys N., Tally M., Andersson G., Georges M., Andersson L. A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature*, 2003, 425 (6960): 832-836 (doi: 10.1038/nature02064).
14. Chang K.C. Key signalling factors and pathways in the molecular determination of skeletal muscle phenotype. *Animal*, 2007, 1(5): 681-698 (doi: 10.1017/S1751731107702070).
15. Estellé J., Mercadé A., Noguera J.L., Pérez-Enciso M., Óvilo C., Sánchez A., Folch J.M. Effect of the porcine IGF2-intron3-G3072A substitution in an outbred Large White population and in an Iberian×Landrace cross. *J. Anim. Sci.*, 2005, 83(12): 2723-2728. (doi: 10.2527/2005.83122723x).
16. Heuven H.C.M., Bovenhuis H., Janss L.L.G., Van Arendonk J.A.M. Efficiency of population structures for mapping of Mendelian and imprinted quantitative trait loci (QTL) in outbred pigs using variance component methods. *Genet. Sel. Evol.,* 2005, **37:** 35-655 (doi: 10.1051/gse:2005019).
17. Oczkowicz M., Tyra M., Walinowicz K., Różycki M., Rejduch B. Known mutation (A3072G) in intron3 of the *IGF2* gene is associated with growth and carcass composition in Polish pig breeds. *J. Appl. Genet.*, 2009, 50(3): 257-259 (doi: 10.1007/BF03195681).
18. Kostyunina O.V., Svezhentseva N.A., Zinov'eva N.A., Dotsev A.V., Shakhina A.V., Sizareva E.I., Gladyr' E.A. Effect of ESR and IGF2 marker genotypes on the breeding values of the large white boars. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2011, 6: 54-59.
19. Kostyunina O.V., Kramarenko S.S., Svezhentseva N.A., Sizareva E.I., Zinov'eva N.A. The association of ***IGF2*** with productive traits of pigs of large white breed in the aspect of sexual differentiation. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2015, 50(6): 736-745 (doi: 10.15389/agrobiology.2015.6.736eng).
20. Crawley J.N., Fiske S.M., Durieux C., Derrien M., Roques B.P. Centrally administered cholecystokinin suppresses feeding through a peripheral-type receptor mechanism. *J. Pharmacol. Exp. Ther.*, 1991, 257: 1076-1080.
21. Asin K.E., Bednarz L. Differential effects of CCK-JMV-180 on food intake in rats and mice. *Pharmacol. Biochem. Be.*, 1992, 42(2): 291-295.
22. Clutter A.C., Sasaki S., Pomp D. Rapid communication: the cholecystokinin type-A receptor (CCKAR) gene maps to porcine chromosome 8. *J. Anim. Sci.*, 1998, 76(7): 1983-1984.
23. Houston R.D., Haley C.S., Archibald A.L., Cameron N.D., Plastow G.S., Rance K.A. A polymorphism in the 5' untranslated region of the porcine Cholecystokinin Type-A Receptor

(CCKAR) gene affects feed intake and growth. *Genetics*, 2006, 174(3): 1555-1563 (doi: 10.1534/genetics.106.059659).
24. Johnson R. Effect of DNA markers in Nebraska selection lines. *Nebraska Swine Reports*, 2010: 44-50. Available https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1260&context=coopext_swine. Accessed May 10, 2018.
25. Mountjoy K.G., Mortrud M.T., Low M.J., Simerly R.B., Cone R.D. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol. Endocrinol.*, 1994, 8(10): 1298-1308.
26. Kim K.S., Larsen N.J., Rothschild M.F. Rapid communication: linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene. *J. Anim. Sci.*, 2000, 78(3): 791-792.
27. Kim K.S., Larsen N., Short T., Plastow G., Rothschild M.F. A missense variant of the melanocortin 4 receptor (MC4R) gene is associated with fatness, growth and feed intake traits. *Mamm. Genome*, 2000, 11(2): 131-135.
28. Hernández-Sánchez J., Visscher P., Plastow G., Haley C. Candidate gene analysis for quantitative traits using the transmission disequilibrium test: the example of the melanocortin 4-receptor in pigs. *Genetics*, 2003, 164(2): 637-644.
29. Stachowiak M., Szydlowski M., Obarzanek-Fojt M., Switonski M. An effect of a missense mutation in the porcine melanocortin-4 receptor (MC4R) gene on production traits in Polish pig breeds is doubtful. *Anim. Genet.*, 2005, 37(1): 55-57 (doi: 10.1111/j.1365-2052.2005.01373.x).
30. Houston R.D., Cameron N.D., Rance K.A. A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected large white pig populations. *Anim. Genet.*, 2004, 35(5): 386-390 (doi: 10.1111/j.1365-2052.2004.01182.x).
31. Chen J.F., Xiong Y.Z., Zuo B., Zheng R., Li F.E., Lei M.G., Li J.L., Deng C.Y., Jiang S.W. New evidences of effect of melanocortin-4 receptor and insulin-like growth factor 2 genes on fat deposition and carcass traits in different pig populations. *Asian-Australian Journal of Animal Science*, 2005, 18(11): 1542-1547 (doi: 10.5713/ajas.2005.1542).
32. Óvilo C., Fernández A., Rodríguez M.C., Nieto M, Silióa L. Association of *MC4R* gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. *Meat Science*, 2006, 73(1): 42-47 (doi: 10.1016/j.meatsci.2005.10.016).
33. Van den Maagdenberg K., Stinckens A., Claeys E., Seynaeve M., Clinquart A., Georges M., Buys N., De Smet S. The Asp298Asn missense mutation in the porcine melanocortin-4 receptor (MC4R) gene can be used to affect growth and carcass traits without an effect on meat quality. *Animal*, 2007, 1(8): 1089-1098 (doi: 10.1017/S1751731107000456).
34. Park H.B., Carlborg O., Marklund L., Andersson L. *Melanocortin-4 receptor* (*MC4R*) genotypes have no major effect on fatness in a Large White × Wild Boar intercross. *Anim. Genet.*, 2002, 33(2): 155-157 (doi: 10.1046/j.1365-2052.2002.00824.x).
35. Bruun C.S., Jшrgensen C.B., Nielsen V.H., Andersson L, Fredholm M. Evaluation of the porcine *melanocortin 4 receptor* (*MC4R*) gene as a positional candidate for a fatness QTL in a cross between Landrace and Hampshire. *Anim. Genet.*, 2006, 37(4): 359-362 (doi: 10.1111/j.1365-2052.2006.01488.x).
36. Jokubka R., Maak S., Kerziene S., Swalve H.H. Association of a melanocortin 4 receptor (MC4R) polymorphism with performance traits in Lithuanian White pigs. *J. Anim. Breed. Genet.*, 2006, 123(1): 12-22 (doi: 10.1111/j.1439-0388.2006.00559.x).
37. Salajpal K., Đikić M., Karolyi D., Šurina J., Matković M., Liker B. Effect of MC4R polymorphism on physiological stress response in pigs. Poljoprivreda, 2007, 13(1): 46-50. Available https://hrcak.srce.hr/16049. Accessed May 10, 2018.
38. Misztal I., Tsuruta S., Strabel T., Auvray B., Druet T., Lee D.H. BLUPF90 and related programs (BGF90). *Proc. 7th World Congress on genetics applied to livestock production*. Montpellier, France, 2002, 28(28-07): 21-22.
39. Dube B., Mulugeta S.D., Dzama K. Genetic relationship between growth and carcass traits in Large White pigs. *S. Afr. J. Anim. Sci.*, 2013, 43(4): 482-492.