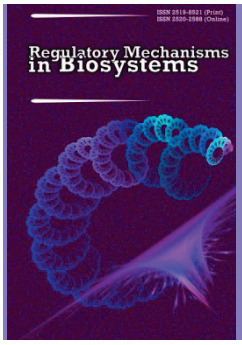

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The influence of feeding level on the growth of pigs depending on their genotype

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The growth and development of pigs is determined by their genotype and environmental conditions (primarily the level of feeding), however, the number of works aimed at studying the complex influence of genetic and non-genetic factors in their interaction is currently insufficient. The purpose of our work was to estimate the effect of the *MC4R* genotype, feeding level and interaction of these factors on growth and backfat thickness of crossbred pigs and to investigate the possibility of correcting the melanocortin-4-receptor gene polymorphism effect by adjusting the ration. Studies were conducted on 50 gilts obtained by crossing sows of the large white breed with landrace boars. Experimental pigs at the "Maxi 2010" farm were weighed at birth, then at the age of 28 days (at weaning) and at the age of 4, 6, 8 months. Fat thickness was measured at the age of 4, 6, 8 months. Genetic studies were conducted in a certified laboratory of Institute of Pig Breeding and Agroindustrial Production. Analysis of 50 blood samples revealed that this group of pigs had a sufficient level of polymorphism for research (Polymorphism Information Content was equil 0.35). The frequency of genotype distribution at the *MC4R* / SNP c.1426 G>A locus was 0.06 (AA) : 0.58 (GA) : 0.36 (GG). The type of feeding significantly influenced the live weight at the age of 4 months and the average daily gains of experimental pigs over the period of 28–120 days. Starting at the age of 6 months a significant effect of the interaction of organized factors (feeding + genotype) was recorded. At the age of 6 months, a significant influence of both the genotype and the level of feeding on the backfat thickness was established. Animals with the GG genotype receiving a restricted feed ration had significantly lower backfat thickness. At the age of 8 months, the difference in backfat thickness between the group with the GG genotype (restricted feed ration) and the AG genotype (high level feeding) reached a value of 12.9% (2.0 mm). Animals with the AG genotype had the lowest performance and the greatest fat thickness under feed limitation, which is important for raising young pigs for subsequent reproduction. Therefore, when selecting pigs to be used for further reproduction, the desired genotype is GG. In the future, it will be desirable to repeat the study on a larger number of pigs, so that the experiment involves a sufficient number of animals with the MC4R AA genotype for statistical processing.

Keywords: pig; feeding technology; DNA-markers; melanocortin 4 receptor gene; genotype-environmental interaction; backfat thickness; average daily gain.

Introduction

Under the same environmental conditions, the growth and fattening productivity of pigs is largely determined by the genotype of the animals (Martins et al., 2020; Suzuki et al., 2021; Óvilo et al., 2022). However, the level of realization of the genetic potential depends on what exactly these environmental conditions are (Davoli & Braglia, 2007; Pierzchała et al., 2012; Soleimani et al., 2021). At the same time, the conditions created on farms are often aimed more at saving resources and reducing costs than at maximizing the genetic potential of animals (Zos-Kior et al., 2020; Brockova et al., 2021). In the scientific literature, cases are described when animals with the desired genotype, under certain conditions, had unexpectedly worse productivity compared to carriers of genotypes associated with lower productivity. For example, in the subtropics, differences that exist between high-yielding and low-yielding breeds in temperate environments are masked by the effects of environmental stressors (Burrow, 2012). Another example is the *ESR1* BB genotype usually associated with a higher number of weaned piglets ($P < 0.05$) compared to the AB genotype (Mencik et al., 2019). This feature of the *ESR1* gene was confirmed in the work of Distl (2007), where there is a mention of the meta-analysis results obtained by Alfonso (2005) which found in 15 studies on more than 9,000 sows that the B allele is superior for the number of born piglets (total and alive). However, in studies conducted by Balatsky et al (2016) on Mirhorod breed sows the opposite trend was found – sows with the ESR1 AA genotype for first farrowing bore 10.63 ± 0.66 piglets, whereas sows with the *ESR1* BB genotype bore 8.83 ± 0.71 piglets. The named authors conclude that under the conditions of their experiment, carriers of the B allele could not realize their genetic potential. Similar results were obtained in the work of Santana et al. (2006), the A allele of the ESR1/PvuII marker had a positive effect on increasing the litter size of piglets in the Brazilian Large White breed sows. Previously, in the study of van Rens et al. (2002), conducted on crossbred sows (Large White x Meishan), the superiority of animals with the ESR1 AB and ESR1 AA genotypes over individuals with the ESR1 BB genotype was established

in terms of the number of piglets. It was concluded that the influence of the ESR1 locus on the reproductive characteristics of pigs must be considered depending on the breed and environmental conditions. The interaction between genetics and nutrition has been studied by quite a lot of scientists (Cameron et al., 2000; Aikins-Wilson et al., 2022; Calta et al., 2022). It should be noted that both the interaction of the additive genotype with feeding (Gourdine et al., 2019; Nagy et al., 2020) and the interaction of the genotype at individual loci with feeding (Wang & Kadarmideen, 2020; Calta et al., 2022; Khalak & Gutyj, 2022) were studied. Augspurger et al. (2002) carried out a feeding experiment in pigs and concluded that different genotypes have different nutrient requirements for growth performances, and differed with regard to feed intake and feed efficiency.

One of the peculiarities of the metabolism of pigs of Ukrainian selection is their propensity for significantly greater accumulation of backfat compared to pigs of the same breeds of European origin (Vashchenko et al., 2019; Sukhno et al., 2022; Vashchenko et al., 2022). Both non-genetic factors, such as pig feeding system (Lebret, 2008; Bankovska et al., 2020) or environmental temperature (Vashchenko & Berezovskyi, 2021) and genetic factors, such as MC4R genotype (Kim et al., 2000; Fan et al., 2009; Loos & Yeo, 2022), influence the intensity of growth and deposition of backfat. Unlike the human melanocortin-4-receptor gene, for which many polymorphic variants have been described, some of which are associated with appetite and obesity (Crovesy & Rosado, 2019; Chiurazzi et al., 2020; Mahmoud et al., 2022), very few polymorphic variants of the MC4R gene have been found in pigs (Fan et al., 2009; Llambí et al., 2020; Bo et al., 2022). A large number of scientists consider p.Asp298Asn to be the most practical melanocortin-4-receptor gene polymorphism (Fan et al., 2009; Gondim et al., 2019; Zhang et al., 2020). However, in some studies, the influence of the MC4R gene on growth rate and backfat thickness was not confirmed (Park et al., 2002; Dvořáková et al., 2011). The contradictory results obtained by various scientists regarding the effect of the melanocortin-4-receptor gene on growth parameters and the formation of backfat became one of the reasons for conducting our research. Another one is the small amount of research on the possibility of correcting the effect of this DNA marker by regulating the level of pig feeding (Calta et al., 2022). As a result, the aim of our research was to find out how the *MC4R* genotype affected growth and backfat thickness of experimental pigs and to establish the possibility of correcting the influence of the melanocortin-4-receptor gene polymorphism by adjusting the diet.

Materials and methods

For genetic studies, 50 blood samples were collected from two-breed crossbred female piglets (Large White × Landrace) of the herd the "Maksi 2010" farm, located in Poltava Region (Ukraine). All experiments were carried out in accordance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasburg, 1985) and the Ukrainian law "On the protection of animals against ill-treatment" No. 3447-IV edited on 04/08/2017. The Committee for the Maintenance and Use of Animals of the Institute of Pig Breeding and Agroindustrial Production gave their approval to the study.

Genetic investigations have been conducted in an Institute of Pig Breeding and Agroindustrial Production accredited laboratory. Genomic DNA was isolated from 200 µl of blood using "Chelex 100" (Walsh et al., 1991). The PCR-RFLP technique was used to type DNA (Hlazko et al., 2001). A fragment of the MC4R gene (MC4R / SNP c.1426 G>A / 2-nd exon / NCBI accession number rs 178554175 / Asp >Asn) consisting of 220 bp was amplified using a pair of specific primers: forward: 5'-TGATTCAGGATCTATTGCTACTA -3' and reverse: 5'-TATACTGTCGCTTGTGCTTAAG -3' (Kim et al., 2006). PCR reactions were performed in 25 µL (final volume) of the mixture containing 10–100 mg of genomic DNA, 200 nM of forward and reverse primers, 2.5 mM $MgCl_2$, 0.25 mM of each of the dNTPs and one unit of the recombinant Taq DNA Polymerase (Thermoscientific, EU). PCR amplification program: 95 °C – 2 minutes; 30 cycles: 95 °C – 30 s, annealing of primers 52 °C – 30 s, 72 °C – 105 s; 72 °C – 7 min. Thermocycler "Tertsyk-2" (DNA Technology, RF) was used to carry out PCR. The amplification fragment of the MC4R gene was restricted with the enzyme Taq I (Thermo Fisher Scientific, Lithuania) at 65 °C – 3 hours, which resulted in restriction fragments appearing that were specific to the following genotypes of the MC4R gene: AA – 220 bp, AG – 220, 150, 70 bp, GG: 150, 70 bp (Fig. 1).

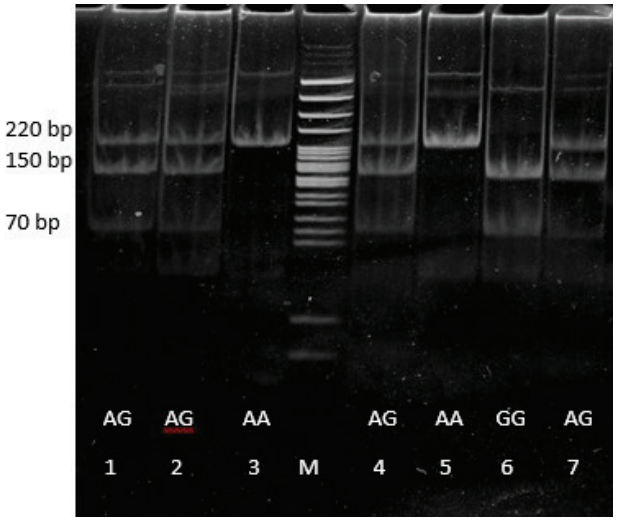

Fig. 1. Electrophoregram of the products of Taq I restriction DNA locus MC4R in a 3.5% agarose gel: *1*, *2*, *4*, *7* – experimental animals with the AG genotype; *3*, *5* – with the AA genotype; *6* – with the GG genotype; *M* is a marker of molecular weight pBR322 DNA-MspI

GenAlEx 6.0 software (Peakall, 2012) was used to calculate the allele frequencies, genotype frequencies, and Polymorphic Information Content (PIC). Reliability of the differences between the observed genotypes frequencies and expected genotypes frequencies was calculated using Chi-square test.

The animals on the farm were fed compound feed balanced according to the norms of feeding breeding animals (Provatorov et al., 2007). Prestarter feed was given to nursing piglets, and it contained 231 grams of crude protein, 11.1 grams of lysine, and 15.4 MJ of metabolic energy per 1 kg of dry matter. Starting from weaning, the piglets were divided into groups (according to the principle of analogues) that received different types of feeding (Table 1).

Table 1
Nutritional value of the daily ration for groups of pigs at different feeding levels

Weight of pigs, kg	Metabolic energy, MJ	Dry matter, kg	Crude protein, g	Lysine, g	Methionine + cystine, g	Crude fiber, g
High feeding level						
20–30	17.6	1.26	239	12.4	7.47	65.5
30–40	19.2	1.37	260	13.5	8.12	71.2
40–50	20.7	1.57	267	14.1	8.49	100.3
50–60	22.4	1.70	289	15.3	9.20	109.1
60–70	23.8	1.80	306	16.2	9.74	115.4
70–80	25.2	1.91	325	17.2	10.31	122.1
80–90	26.0	2.10	336	17.8	10.74	170.2
90–130	29.8	2.40	384	20.4	12.25	194.1
Restricted feeding level						
20–30	19.4	1.39	263	13.6	8.22	72.1
30–40	21.1	1.51	286	14.9	8.93	78.3
40–50	22.8	1.73	294	15.5	9.34	110
50–60	24.6	1.87	318	16.8	10.12	120
60–70	26.2	1.98	337	17.8	10.71	127
70–80	27.7	2.10	358	18.9	11.33	134
80–90	28.6	2.31	370	19.6	11.77	187
90–130	32.8	2.64	422	22.4	13.42	213

Experimental pigs were weighed at birth, at the age of 28 days (at weaning), at the age of 4, 6 and 8 months. Piglets were weaned from the sow when they reached the age of 28 days. A portable digital Renco Lean-Meter (Renco Corporation, USA) was used to measure the thickness of the backfat at the level of the 6th-7th ribs (Zhang et al., 2018). The measurement was carried out when the pigs reached the age of 4, 6

and 8 months. Conversion of backfat thickness for live weight of 50, 85 and 120 kg was carried out using the linear regression equation: $BF = BF_i + \Delta m \times b$, where BF_i – actually measured value of backfat thickness, Δm – difference between actually measured weight and calculated weight, b – regression coefficient reflecting the change in backfat thickness depending on live weight. The average daily gains were calculated for periods from 28 days to 4 months, 4–6, 6–8 months and from birth to 8 months.

Data processing was performed using software Statistica 10 (StatSoft, EU). The tables show the arithmetic mean values and their standard errors (x ± SE). The significant of differences between the genotypes was assessed using two-factor analysis of variance (ANOVA). Fisher's F-test was used to assess the ratio of intergroup and intragroup variability. Tukey's HSD test was used to test for significant differences in multiple comparisons. Differences were viewed as significant at $P < 0.05$.

Results

As a result of our genetic population analysis of the MC4R c.1426 G>A SNP marker for experimental two-breed pigs, the distribution of allele frequencies and genotypes was obtained (Table 2). For *MC4R* / SNP c.1426 G>A, both alleles of the gene *MC4R* – A and *MC4R* – G were detected in the studied herd. The frequency of the allele G is significantly (1.86 times; $P < 0.001$) higher compared to the frequency of the allele A. When calculating the population genetic characteristics, no significant deviation of the genotype frequencies according to the equilibrium Hardy-Weinberg law for the *MC4R* gene was found. In the studied micropopulation for *MC4R* / SNP c.1426 G>A, the frequency of the AG genotype prevailed over both homozygous genotypes GG and AA. Also, the value of the fixation index indicates the predominance of heterozygous genotypes in the studied population. The homozygous AA genotype accounted for the smallest proportion in frequency.

Table 2
Distribution of frequencies of alleles and genotypes by gene in experimental pigs (n = 50)

Locus / polymorphism	Allelic frequency	Genotypes frequencies			χ^2	Fixation index (F)
		AA	AG	GG		
MC4R / SNP c.1426 G>A	A = 0.35 G = 0.65	0.06 (0.12)	0.58 (0.46)	0.36 (0.42)	3.774	–0.275

Notes: in parentheses are the expected genotypes' frequencies found out according to the Hardy-Weinberg equilibrium; to figure out the significance of the actual genotype frequency distribution's divergence from the expected one, values of χ^2 were calculated.

According to the level of locus variability, no significant deviation of the level of actual heterozygosity (He = 0.580) from the theoretically expected one (Ho = 0.455) was found. However, the polymorphism level of the *MC4R* / SNP c.1426 G>A locus required for association studies is at the optimal level of PIC = 0.35 (Polymorphism Information Content). According to Botstein et al. (1980), the optimal PIC indicator for association studies, which provides the necessary diversity of genotypes to establish their relationships with productivity indicators, is a value from 0.25 to 0.75 units.

The effects of genotype and feeding level on the live weight and backfat thickness of growing pigs at the age of 4, 6, and 8 months was analyzed (Table 3). Since there were only three animals with the AA genotype, it was not possible to divide them into groups with different feeding levels, so these three pigs were not used in further studies.

Table 3
Influence of feeding level and genotype on growth and backfat thickness of pigs (x ± SE)

Productive traits	High level of pig feeding		Restricted level of pig feeding		Feed level effect		Genotype effect		Interaction feed level and genotype	
	Genotype		Genotype		F	P	F	P	F	P
	AG (n = 15)	GG (n = 9)	AG (n = 14)	GG (n = 9)						
Weight at birth, kg	1.313 ± 0.024	1.269 ± 0.044	1.272 ± 0.025	1.251 ± 0.028	1.18	0.283	1.21	0.277	0.14	0.707
Weight at 28 days, kg	8.033 ± 0.077	8.157 ± 0.163	7.943 ± 0.096	7.799 ± 0.124	3.13	0.084	0.01	0.918	1.44	0.237
Weight at 4 months, kg	49.68 ± 0.52	49.79 ± 0.89	48.10 ± 0.48	48.50 ± 0.35	6.60	0.014	0.15	0.701	0.06	0.806
Weight at 6 months, kg	87.01 ± 1.07^a	85.91 ± 0.84^a	82.41 ± 0.41^b	85.59 ± 0.21^a	13.66	0.001	1.36	0.251	6.74	0.013
Weight at 8 months, kg	125.73 ± 0.77^a	123.17 ± 0.30^b	118.45 ± 0.41^c	122.27 ± 0.39^b	0.69	0.409	68.8	$3.82*10^{-6}$	28.32	$2.78*10^{-5}$
Backfat thickness at 4 months (conversion per 50 kg of live weight kg), mm	10.31±0.15	10.26±0.12	10.24±0.13	9.93±0.20	1.16	0.287	1.24	0.271	0.62	0.436
Backfat thickness at 6 months (conversion per 85 kg of live weight kg), mm	$13.77±0.22^a$	$13.17±0.21^{ab}$	$13.42±0.17^a$	$12.40±0.31^b$	5.14	0.029	12.5	0.001	0.80	0.376
Backfat thickness at 8 months (conversion per 120 kg of live weight kg), mm	$17.49±0.25^a$	$16.33±0.27^{bc}$	$16.59±0.18^b$	$15.49±0.39^c$	11.21	0.002	17.8	$1.22*10^{-4}$	0.01	0.908
Average daily gain from 28 days to 4 months, g	452.7 ± 5.6	452.5 ± 8.0	436.5 ± 4.7	442.4 ± 4.4	5.86	0.020	0.19	0.662	0.27	0.609
Average daily gain for the period 4–6 months, g	622.2 ± 13.2^a	602.0 ± 15.3^{ab}	571.8 ± 3.5^b	618.1 ± 4.3^a	5.52	0.023	1.26	0.269	9.30	0.004
Average daily gain for the period 6–8 months, g	645.3 ± 13.5^a	620.9 ± 10.5^{ab}	600.7 ± 4.7^b	611.3 ± 4.3^{ab}	0.55	0.464	9.52	0.004	2.83	0.100
Average daily gain from birth to 8 months, g	518.4 ± 3.2^a	507.9 ± 1.2^b	488.2 ± 1.6^c	504.2 ± 1.7^b	0.80	0.378	68.4	$3.81*10^{-6}$	28.34	$2.78*10^{-5}$

Note: different letters within each row indicate significant differences between groups according to the Tukey's HSD test results.

The results of weighing at birth and at 28 days showed that at the beginning of the research, when the groups were formed, there was no significant difference between the experimental groups (Table 3). The type of feeding significantly influenced the live weight at the age of 4 months and the average daily gains of experimental pigs over the period of 28–120 days. With increasing age, at 6 months, in addition to the significant influence of the type of feeding on weight and growth, a significant effect of the interaction of organized factors (feeding + genotype) was recorded. The influence of the interaction of factors was manifested in growth retardation and reduced live weight in pigs with the AG genotype on a limited feeding ration. Compared to the group with the AG genotype (high level of feeding), the live weight was lower by 4.6 kg, or 5.58%. Compared to the group with the GG genotype (high level of feeding), the difference was 3.5 kg, or 4.25%, and compared to the group with the GG genotype (limited feeding), the live weight was less by 3.2 kg, or 3.88%.

At the age of 6 months, a significant influence of both the genotype and the level of feeding on the backfat thickness was established. Animals with the GG genotype receiving a restricted feed ration had significantly less backfat thickness compared to pigs with the AG genotype by 1.4 mm (high feeding) and 1.0 mm (restricted feeding), respectively, or by 11.3% and 8.1%.

At the age of 8 months, the difference in backfat thickness between the group with the GG genotype (restricted feed ration) and the AG genotype (high level feeding) became even larger and reached a value of 12.9% (2.0 mm). The difference in backfat thickness between the group with the GG genotype receiving a restricted ration and the group with the same genotype but on a high level of feeding was 1.1 mm or 7.1%.

Despite the fact that the difference in average daily gains for the period from weaning to 4 months between groups with the AG genotype and different levels of feeding amounted to 16.2 g (3.7%), it turned out to be insignificant. A significant difference between the average daily gains of the groups was found for the period from 4 to 6 months. With limited feeding, the group of pigs with the AG genotype had a worse average daily gain compared to animals carrying the GG genotype by 46.4 g or

7.5%. An even greater difference (50.4 g or 8.2%) was found between the AG (restricted feeding) and AG (increased feeding) groups.

From 6 to 8 months, a significant difference in average daily gains was established only in pigs with the AG genotype between groups with different levels of feeding. Average daily gains in AG animals fed a high-energy diet were 44.6 g (7.3%) higher than in pigs fed a restricted diet.

In general, during the entire growing period (from birth to 8 months of age), the highest average daily gains were recorded in the group of pigs with the AG genotype, in animals that received a high level of feeding. This group was better than the counterparts who received the same diet, but had the GG genotype by 2.1% (10.5 g). Compared to the GG (restricted diet) group, the AG (high level of feeding) group had 2.8% (14.2 g) greater daily gain. However, the largest difference in average daily gain was observed between the AG (high level of feeding) and AG (limited level of feeding) groups – 6.0%, or 30.2 g.

It should be noted that a reliable influence of feeding on the difference in average daily gain was detected at an earlier age, from 28 days to 6 months. Whereas, starting from six months of age, the influence of genotype on average daily growth was established.

Discussion

In our studies, it was established that the frequency of the *MC4R*-A allele is 1.86 times ($P < 0.001$) lower in animals of the Large White × Landrace cross compared to the *MC4R*-G allele. This is consistent with the data of other researchers who found that the allele *MC4R*-G is more common in modern pig breeds and their crosses, which have been improved to achieve better growth and a lean meat deposition (Burgos et al., 2006; Galve et al., 2012; Szyndler-Nędza et al., 2013). The German origin Pietrain sire population had more than 90% GG homozygotes and extremely low backfat thickness (Burgos et al., 2006). In the crosses obtained as a result of different variants of the combination of the Great White, Landrace, Duroc, Pietren and Hampshire breeds, the frequency of the *MC4R*-G allele was 0.62, the frequency of the MC4R-A allele was 0.38 (Calta et al., 2022). In contrast, in aboriginal breeds with a large thickness of the back fat the *MC4R*-A allele prevails (Vashchenko et al., 2019; Wang et al., 2019).

The AG genotype was the most common in White × Landrace experimental pigs. There were 1.61 times more heterozygous animals compared to GG homozygotes and 9.67 times more compared to AA homozygotes. Similar results were obtained in the studies of Calta et al. (2022), where GA genotype was detected in 50.0% of experimental pigs (251 pigs), and the smallest number was of AA genotype pigs (111 head, or 22.1%). In the research of Stachowiak et al. (2006) it was established that the most common genotype in experimental animals of the Polish Large White breed was AA (0.60), while in the Polish Landrace breed the most common genotype was GG (0.51). Somewhat different results on the distribution of genotypes were established in another study carried out on the same breeds (Piórkowska et al., 2010). The frequencies of AA : AG : GG genotypes in the Polish Large White breed were 32.7% : 44.8% : 22.5%, respectively, and in the Polish Landrace – 6.5% : 34.3% : 59.2%. However, although the frequency of genotypes in the Polish Great White breed is slightly different in this study, the common feature is the highest frequency of the GG genotype in Polish Landrace pigs. Similar results were obtained when analysing the frequency of alleles of the MC4R gene in the Italian Great White and Italian Landrace breeds (Davoli et al., 2012). It was found that in the Italian Large White breed, the A allele is most frequent (0.694), while on the contrary, in the Italian Landrace, the G allele has the highest frequency (0.812). The most plausible explanation for the fact is that the crosses of the Large White and Landrace breeds have largest number of animals with the heterozygous genotype AG. The value of the fixation index obtained in our studies ($F = -0.275$) confirmed that the highest frequency of heterozygotes is the result of selection of couples for artificial insemination. To compare the value of the fixation index, we can refer to the results of determination of the fixation index in 19 European breeds (Bovo et al., 2020). In that study, the average value of F_{st}, depending on the breed, ranged from 0.086 to 0.199. The value obtained by us indicates an extremely high level of heterozygosity in experimental pigs, which is explained by their origin.

At the beginning of the research, when piglets were weaned at the age of 28 days, no significant difference was found between groups with *MC4R* AA and *MC4R* GG genotypes. Conducting such an analysis was important because in studies (Canario et al., 2010) it was established that genetic factors can affect the live weight of piglets at the age of 21–30 days. The absence of a significant difference between groups with different genotypes determined the possibility of further research.

A significant influence of the type of feeding on pigs live weight and average daily gains ($P < 0.05$) was found at the age of 4 months, two months earlier than the effect of genotype on these characteristics was revealed. A significant effect of genotype on fat thickness was also found only after the experimental pigs reached the age of six months. Such results are consistent with the data obtained in the studies of Salajpal et al. (2009) and Calta et al. (2022), where it was established that a significant influence of the MC4R gene polymorphism is observed only in pigs slaughtered when they reach a high live weight (more than 100 kg). This can be explained by the fact that in the early postnatal period, muscle tissue grows more intensively compared to adipose (Rudar et al., 2019).

It was established that at the age of 8 months, at a high level of feeding, pigs with the AG genotype achieve a higher live weight compared to other groups. Conversely, pigs with the same genotype but under conditions of limited feeding had the worst live weight values at the same age. Similar results were obtained in the studies of Calta et al. (2022), pigs with the AA genotype when fed ad libitum had the best average daily gains, and those with restricted feeding had the worst energy utilization and lower live weight.

Pigs with the GG genotype have a thinner back fat starting at six months of age. This is confirmed by the results of not only our study, but also the results of the studies of many other researchers (Xiao-Hui et al., 2008; Galve et al., 2012; Xu et al., 2022). The fact that the difference between groups in fat thickness is manifested at the age of 6 months and older is consistent with the statement that intensive growth of adipose tissue begins at later stages of ontogenesis compared to muscle tissue (Campbell & Dunkin, 1982; Virgili et al., 2003).

The significant influence of the interaction of feeding and genotype factors on the average daily gains of pigs is determined by the fact that pigs with the GA genotype have greater gains due to a greater consumption of feed, which is deposited in the form of adipose tissue. In the absence of an excess amount of feed, animals with the specified genotype have lower growth rates compared to the GG genotype. This is explained by a worse feed conversion, which in turn is caused by the formation of a larger amount of adipose tissue in animals with the GA genotype, hence increased fat in the carcass requires more feed during pig growth (Burgos et al., 2012). Such conclusions are consistent with the studies of Calta et al. (2022), who believe that high growth energy can be obtained from pigs with the AA genotype only if they receive a diet with a high level of nutrition. Calta et al. (2022) also concluded that pigs with the AA genotype use available feed less efficiently. However, in contrast to the results obtained in our studies, in the experiments of Calta et al. (2022), pigs with the GA genotype had better gains compared to the GG genotype. The possible reason for the differences in the results may be some difference in the feeding of the experimental animals, as well as possible differences in the genotype of other genes associated to growth. Different results than in our studies were obtained in studies of pigs with a different *MC4R* genotype on the Lithuanian white breed (Jokubka et al., 2006). The authors of that paper concluded that pigs with the A allele can change their growth pattern under restricted feeding and produce more muscle and less fat. In our opinion, such differences in the results are explained by the different genetic origin of the experimental pigs.

It is worth noting that the thickness of lard was influenced by the genotype and level of feeding as separate factors, the influence of the interaction of these two factors was not detected. Therefore, it is possible to adjust the thickness of backfat in pigs by feeding regardless of their genotype at the locus *MC4R* / SNP c.1426 G>A.

Conclusions

In the Large White × Landrace crossbred pigs, the AG genotype has the highest frequency in the *MC4R* / SNP c.1426 G>A locus, and the AA

genotype has the lowest frequency. Allele G occurs 1.86 times more often compared to allele A. The significant difference between the frequencies of alleles can probably be explained by the selection of meatier animals with GG genotype for reproduction in the parental herd.

The level of polymorphism in the experimental population of Large White × Landrace pig cross was high enough to conduct an associative analysis (Polymorphism Information Content was equal to 0.35 at its optimal level of 0.25–0.75). The influence of genotype and feeding level on growth indicators and fat thickness in the Large White × Landrace crossbred pigs is manifested at the age of 4–6 months. At a high level of feeding, animals with the GA genotype were characterized by the highest live weight and the best gains, but this gain was obtained due to the growth of adipose tissue, as evidenced by the value of the fat thickness, which was the largest in this group. Under conditions of feed restriction, which is necessary for growing young pigs for further reproduction, animals with the AG genotype had the worst growth and the largest fat thickness. Consequently, the preferred genotype when choosing pigs to be used for subsequent reproduction is GG. In future studies, it will be desirable to use a larger number of pigs and investigate the influence of the *MC4R* AA genotype on the growth and development traits of pigs, as well as its interaction with the level of feeding.

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