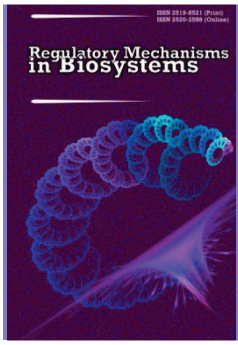

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(2), 312–318
doi: 10.15421/022346

The influence of genotype and feeding level of gilts on their further reproductive performance

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Article info

Received 13.04.2023
Received in revised form 18.05.2023
Accepted 27.05.2023

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Zhukorskyi, O. M., Tsereniuk, O. M., Sukhno, T. V., Saienko, A. M., Polishchuk, A. A., Chereuta, Y. V., Shaferivskyi, B. S., & Vashchenko, P. A. (2023). The influence of genotype and feeding level of gilts on their further reproductive performance. Regulatory Mechanisms in Biosystems, 14(2), 312–318. doi:10.15421/022346

Since the efficiency of pig breeding largely depends on the reproductive ability of sows, one of the promising areas of research in animal husbandry is the study of factors whose correction can improve the fertility and quality of the offspring. Our work purpose was to investigate the effect of gilts' feeding level, MC4R genotype and interaction of these factors on the reproductive traits pigs after reaching puberty. The object of the study was crossbred pigs obtained as a result of the combination of sows of the Large White breed with boars of the Landrace breed. To carry out the research, the animals were divided into four groups that differed among themselves by the genotype of the melanocortin 4 receptor gene and the level of feeding of the experimental animals. To determine the genotype, the PCR-RFLP method was utilized. Genetic studies were conducted in a certified laboratory of the Institute of Pig Breeding and Agroindustrial Production. The reproductive capacity of the pigs was determined after receiving the farrowing by the following traits: piglets born alive per farrowing (animals), weight of litters of piglets at birth (kg), average weight of piglet at birth (kg), number of weaning piglets (animals), weight of litters of piglets at 28 days (kg), average weight of piglet at 28 days (kg), average daily gain of suckling piglets from 0 to 28 days (g). It was established that the traits of reproductive ability of crossbred Large White × Landrace pigs were affected by the interaction of genotype and feeding factors. When limiting the nutritional value of the daily diet, sows with the AG genotype outperformed those with the GG genotype by 1.2 piglets born alive per farrowing, by 3.0 kg for litter weight at birth, and by 0.1 kg for the average weight of a piglet at birth. The advantage of the AG genotype for average piglet weight at 28 days was even larger. Conversely, in conditions of a high level of feeding, animals with the GG genotype had higher productivity in terms of such traits as piglets born alive per farrowing – by 1.6 animals; weight of litters of piglets at birth – by 4.1 kg; for average weight of piglet at birth – 0.2 kg; by number of weaning piglets – 2.0 animals; by weight of litters of piglets at 28 days – 28.8 kg; for average weight of piglet at 28 days – 1.1 kg and for average daily gain from 0 to 28 days – 34.6 g. In the future, the goal is to develop a system of differentiated rationing of pigs' diets depending on their genotype.

Keywords: pig; restricted feeding; DNA-marker; melanocortin 4 receptor gene; genotype-environmental interaction; growth; reproductive traits.

Introduction

Saving resources, reducing costs and increasing the efficiency of using animal organisms to minimize the negative impact on the environment during the production of livestock products is becoming a global problem of our time (Zos-Kior et al., 2020; Brockova et al., 2021). In order to be able to influence the productivity of pigs and increase efficiency of pork production, it is first necessary to analyze the factors related to the growth performance of pigs from birth to slaughter (Douglas et al., 2013; Camp Montoro et al., 2020). According to scientific papers, both genetic (Mohammadabadi et al., 2021; Malgwi et al., 2022; Vashchenko et al., 2022) and non-genetic (Chakurkar et al., 2021; Zappaterra et al., 2022; Vashchenko et al., 2023) factors affect the growth and development of piglets. This also applies to the reproductive capacity of sows (Vashchenko et al., 2022).

The influence of feeding is the most significant among other non-genetic factors (Carcò et al., 2018; Herrera-Cáceres et al., 2020). There are many works that have studied this effect. For illustrate, in one experiment (Schiavon et al., 2018), a half of the experimental pigs received restricted amounts of feed throughout the entire developing phase, while the other half were fed ad libitum. Performance during the growing and finishing phases was impacted by feeding level. In comparison to pigs fed ad libitum, restricted-diet pigs consumed less feed during the growing, finishing, and overall periods ($P < 0.001$, $P = 0.002$ and $P < 0.001$, respectively), grew 5% less overall ($P = 0.014$) and 7% less during the finishing period ($P = 0.033$), but were more productive during the growing and overall periods ($P = 0.050$). They also lost less weight after 14 hours of pre-slaughter fasting ($P = 0.014$) and were 3% lighter at the end of the research ($P = 0.018$).

Also the type of feeding is an important factor affecting the production efficiency and biosafety of feed in pig farming (Jiang et al., 2019). According to monitoring data, liquid feeding improves gut health and lowers the number of cases of Salmonella. The incidence of sub-clinical *Salmonella* infection was found to be ten times lower on farms using liquid feeding than on farms using dry compound diets in a study of 320 farms in Holland. Pigs fed liquid diets usually had improved daily liveweight gain and feed conversion ratio, according to recent studies comparing the performance of pigs fed dry or liquid diets (Brooks et al., 2001).

Another important factor affecting the growth of pigs is the temperature of the environment. The impact of heat stress on swine growth and feed efficiency is influenced by both temperature and body mass. Due to the impact of feed restriction on the composition of body weight gain (more lean/less fat), feed efficiency typically rises for mild heat stress. Finishing pigs kept at a temperature greater than 30 °C have been found to have less feed efficiency. This decline in feed efficiency is due to a decreased amount of ingested energy that is accessible for tissue growth, which is primarily explained by a significant decrease in feed intake. There is no doubt that heat stress decreases facility and operational efficiency (amount of carcass weight produced per barn per year), as it significantly shortens the time it takes for animals to achieve market weight, independent of the nuances within the feed efficiency equation (Mayorga et al., 2019).

Research has established that the factors that affect the growth of young pigs, in the vast majority of cases, also affect the reproductive capacity of sows and the quality of the offspring if these factors were active during the fertile period (Carrión-López et al., 2022). According to other researchers, the reproductive traits of sows can be influenced by the growth factors of young pigs, which acted in the early stages of their ontogenesis (Zubenko, 2012). The time of appearance of the first estrus, productive indicators of farrowings and the duration of the sow's productive life depend on the intensity of growth during the rearing of gilts intended to replace sows (Małopolska et al., 2018).

Genetic factors can be isolated for research using genotype information from DNA markers associated with pig performance. A fairly well-studied SNP-marker affecting feed intake, growth rate and fat thickness of pigs is a polymorphism in the melanocortin 4 receptor gene (MC4R / SNP c.1426 G>A), which causes the substitution of the amino acid asparagine for aspartame in the structural protein, which in turn, disrupts the neuro-humoral regulation of food intake and affects the growth and development of adipose tissue (Llambí et al., 2020; Zeng et al., 2022).

When conducting research on the influence of various factors on the productivity and reproductive capacity of pigs, it is necessary to take into account the interaction of genetic and non-genetic factors. Genotypes that contribute to better performance under the same conditions can have a negative effect when the feeding conditions are changed. For example, pigs with the MC4R AG genotype had better live weight compared to MC4R GG genotype carriers when fed a diet high in energy, protein and essential amino acids, but performed significantly worse under restricted feed conditions (Vashchenko et al., 2023). In other studies, pigs with the same genotype (MC4R AA) had the best average daily gains when fed ad libitum, and the worst gains and feed conversion when fed a restricted diet (Calta et al., 2022).

Since no studies were found in the available literature on the study of the influence of the interaction of the melanocortin 4 receptor genotype and the level of feeding of breeding pigs on their subsequent reproductive capacity, the purpose of our study was to investigate the effect of gilts' feeding level, MC4R genotype and interaction of these factors on pigs' reproductive traits after reaching puberty.

Materials and methods

The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1985) and the Order of the First National Congress of the Bioethics (Kyiv, 2001), as well as the law of Ukraine "On the protection of animals against ill-treatment" No. 3447-IV as of 21/02/2006 last amended on 04/08/2017 were followed in every investigation. The study was approved by the Committee for the Maintenance and Use of Animals of the Institute of Pig Breeding and Agroindustrial Production. During the experiment, restrictions on the ability of experimental animals to satisfy their physiological and ethological needs were reduced to a possible minimum. Fixed maintenance of sows was applied only as necessary during estrus, as well as after farrowing during lactation. The state of health of the animals was regularly and thoroughly examined.

The object of the study was crossbred pigs obtained as a result of the combination of sows of the Large White breed with boars of the Landrace breed belonging to the farm "Maxi 2010" located in Poltava district of Poltava region of Ukraine. The subject of the study was the relationship between the level of feeding, polymorphism of the MC4R DNA marker and traits of the reproductive capacity of pigs (number of piglets in a litter born alive, litter weight of piglets, average weight of one piglet in a litter, survival of piglets until weaning). The experiment was organized according to the scheme (Fig. 1).

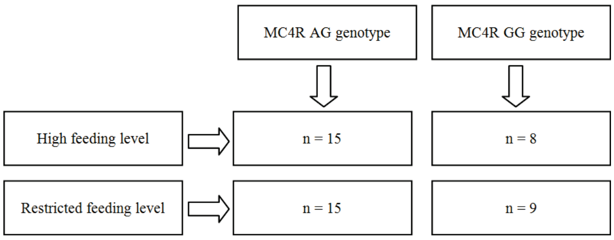

Fig. 1. Research organization scheme (number of animals at groups)

The farm's animals were fed balanced compound feed in accordance with the standards for feeding breeding animals (Provatorov et al., 2007): gilts from two groups received a limited diet that varied depending on the live weight of the animals: after weaning, at a live weight of 20–30 kg, they were fed feed containing per day 17.6 MJ of exchangeable energy, 1.26 kg of dry matter, 239 g of crude protein and 12.4 g of lysine; with the same live weight, the group receiving the enhanced diet consumed 19.4 MJ, 1.39 kg, 263 g and 13.6 g of exchangeable energy and nutrients per day, respectively. During the entire growing period pigs from experimental groups with a high level of feeding received 10% more energy and 12% more crude protein in their daily diet. During the growing period from 90 to 130 kg of live weight, animals from groups with limited feeding received 29.8 MJ of exchangeable energy, 2.40 kg of dry matter, 384 g of crude protein and 20.4 g of lysine; and the animals from the groups receiving the enhanced diet consumed 32.8 MJ of exchangeable energy, 2.64 kg of dry matter, 422 g of crude protein and 22.4 g of lysine per day, respectively. Upon reaching a live weight of 125–130 kg and the appearance of signs of estrus, sows were placed in individual stalls (Fig. 2), where they were artificially inseminated with the sperm of terminal boars of the breeding line PIC 408.

Fig. 2. Maintenance of pigs during the period of estrus in stalls for artificial insemination at Maxi 2010 LLC

Farrowing gilts were kept in group stalls of 4–6 animals each. After insemination, farrowing pigs were fed with a ration according to their weight and norms of feeding breeding animals (Provatorov et al., 2007). Basic indicators of rations for different groups are shown in Table 1.

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

Feeding level	Weight of pigs, kg	Metabolic energy, MJ	Dry matter, kg	Crude protein, g	Lysine, g	Methionine + cystine, g	Threonin, g	Tryptophan, g	Crude fiber, g	Calcium, g	Phosphorus, g
Restricted feeding level	100–120	19.7	1.7	238	10.7	6.3	7.0	1.9	238	14.8	12.2
	121–140	22.0	1.9	266	12.0	7.1	7.8	2.2	266	16.5	13.7
	141–160	24.4	2.1	294	13.2	7.8	8.6	2.4	294	18.3	15.1
	161–180	26.6	2.3	321	14.4	8.5	9.4	2.6	321	19.9	16.5
	181–200	28.7	2.5	346	15.6	9.2	10.1	2.8	346	21.5	17.8
	201–240	29.8	2.6	360	16.2	9.6	10.5	2.9	360	22.4	18.5
	241 and higher	31.0	2.7	374	16.8	9.9	10.9	3.0	374	23.2	19.2
High feeding level	100–120	21.7	1.9	267	12.0	7.1	7.7	2.1	262	16.3	13.4
	121–140	24.2	2.1	298	13.4	7.9	8.6	2.4	293	18.2	15.1
	141–160	26.8	2.3	329	14.8	8.7	9.5	2.6	323	20.1	16.6
	161–180	29.3	2.5	360	16.1	9.5	10.3	2.9	353	21.9	18.2
	181–200	31.6	2.7	388	17.5	10.3	11.1	3.1	381	23.7	19.6
	201–240	32.8	2.8	403	18.1	10.7	11.6	3.2	396	24.6	20.4
	241 and higher	34.1	2.9	419	18.8	11.1	12.0	3.3	411	25.5	21.1

Blood samples were taken from 50 pigs for animal typing by the melanocortin 4 receptor gene. In a certified laboratory of Institute of Pig Breeding and Agroindustrial Production, genetic studies were carried out. With "Chelex 100" (Bio-Rad Laboratories, Inc., USA) genomic DNA was extracted from 200 µL of blood (Walsh et al., 1991). To type DNA, the PCR-RFLP method was utilized (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. PCR was performed in thermocycler "Tertsyk-2" (DNA Technology, RF). The amplification fragment of the MC4R gene was restricted with the enzyme Taq I (Thermo Fisher Scientific, Lithuania) at 65 °C – 3 hours, which caused the appearance of restriction fragments corresponding to the following genotypes of the MC4R gene: AA – 220 bp, AG – 220, 150, 70 bp, GG: 150, 70 bp (Fig. 3).

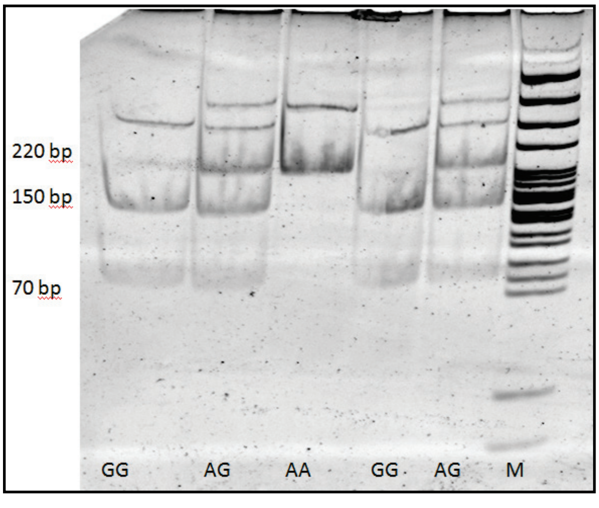

Fig. 3. Electrophoregram of the products of Taq I restriction DNA locus MC4R in a 3.5% agarose gel: *2*, *5* – experimental animals with the AG genotype; *3* – with the AA genotype; *1*, *4* – with the GG genotype; M is a marker of molecular weight pBR322 DNA-MspI

GenAlEx 6.0 software (Peakall, Australia, 2012) was used to calculate the allele frequencies, genotype frequencies, and Polymorphic Information Content (Peakall, 2012). The Chi-square test was used to determine the reliability of the discrepancies between the observed genotype frequencies and expected genotype frequencies.

The reproductive capacity of pigs was determined after receiving the farrowing by the following traits: piglets born alive per farrowing (animals), weight of litters of piglets at birth (kg), average weight of piglet at birth (kg), number of weaning piglets (animals), weight of litters of piglets at 28 days (kg), average weight of piglet at 28 days (kg), average daily gain of suckling piglets from 0 to 28 days (g).

In addition, the possible prolonged effect of different levels of feeding sows before farrowing on the growth of piglets from the moment of weaning to the age of 90 days was investigated, for this the following traits were determined: average weight of piglets at 60 days (kg), average daily gain from 28 to 60 days (g), absolute gain from 28 to 60 days (kg), relative gain from 28 to 60 days (%), average weight of piglets at 90 days (kg), average daily gain from 60 to 90 days (g), absolute gain from 60 to 90 days (kg), relative gain from 60 to 90 days (%).

Utilising software SAS/STAT(R) 15.1 (SAS Institute Inc., USA, 2018), data processing was carried out. The arithmetic mean values and associated standard errors (x ± SE) are displayed in the tables. The significant of differences between the groups was established using two-factor analysis of variance (ANOVA). To evaluate the proportion of intergroup to intragroup variability, Fisher's F-test was utilised. To look for significant differences across multiple comparisons, Tukey's HSD test was applied, differences were considered significant when $P < 0.05$.

Results

As a result of carrying out laboratory genetic and molecular studies, the genotypes of experimental crossbred pigs were determined according to the melanocortin 4 receptor gene (MC4R c.1426 G>A SNP). Distribution of genotypes was: AA – 3 animals, GA – 17 animals, GG – 30 animals. Allelic frequency was: A = 0.35 and G = 0.65. The actual and expected frequency of the genotypes (given in parentheses, determined using Hardy-Weinberg's equilibrium) is AA – 0.06 (0.12); AG – 0.58 (0.46); GG – 0.36 (0.42). In comparison to the frequency of the allele A, the frequency of the allele G was 1.86 times more ($P < 0.001$). The prevalence of the AG genotype outweighed both homozygous genotypes GG and AA in the micropopulation for MC4R / SNP c.1426 G>A under study. Additionally, the fixation index value shows that heterozygous genotypes predominate in the population under study. The homozygous AA genotype has the lowest frequency frequency contribution. Since three is too small a number of animals to be divided into two groups, we used only pigs with GA and GG genotypes for further studies. The required polymorphism level for association studies at the MC4R / SNP c.1426 G>A locus was established Polymorphism Information Content (PIC), which was equal 0.35.

After the test gilts were inseminated with the sperm of terminal boars of the PIC 408 line, farrowings were obtained from them. Based on the results of farrowing, the influence of genotype and feeding level on reproductive capacity of sows was determined (Table 2). As we can see, the influence of the interaction of organized factors (feeding and genotype) was significant for all studied productive features. The effect of the level of feeding was established for the traits measured after the end of the suckling period. At a high level of feeding, sows with the GG genotype performed better, while at limited feeding, sows with the GA genotype were better (Table 3).

Table 2
The significance of the influence of organized factors (feeding and genotype) on reproductive traits of sows and growth of their offspring

Productive traits	Feeding effect		Genotype effect		Interaction effect	
	F	P	F	P	F	P
Piglets born alive per farrowing, animals	0.444	0.509	1.038	0.314	13.017	0.001
Weight of litters of piglets at birth, kg	0.948	0.336	3.303	0.076	32.653	$1.18*10^{-5}$
Average weight of piglet at birth, kg	0.457	0.502	3.309	0.076	21.230	$9.87*10^{-5}$
Number of weaning piglets, animals	5.764	0.021	0.135	0.715	4.412	0.042
Weight of litters of piglets at 28 days, kg	9.959	0.003	0.778	0.383	21.592	$9.02*10^{-5}$
Average weight of piglet at 28 days, kg	5.231	0.027	4.576	0.038	62.488	$4.95*10^{-7}$
Average daily gain of piglets from 0 to 28 days, g	5.899	0.019	3.622	0.064	59.133	$2.43*10^{-6}$

Table 3
Characteristics of the reproductive capacity of sows depending on their genotype and the level of their feeding (x ± SE)

Productive traits	High level feeding		Restricted feeding	
	AG (n = 15)	GG (n = 9)	AG (n = 14)	GG (n = 9)
Piglets born alive per farrowing, animals	11.27 ± 0.40^{a}	12.89 ± 0.35^{b}	12.71 ± 0.35^{b}	11.56 ± 0.29^{ab}
Weight of litters of piglets at birth, kg	14.22 ± 0.55^{a}	18.32 ± 0.87^{b}	18.05 ± 0.53^{b}	15.03 ± 0.48^{a}
Average weight of piglet at birth, kg	1.263 ± 0.024^{a}	1.419 ± 0.044^{bc}	1.422 ± 0.025^{b}	1.301 ± 0.028^{ac}
Number of weaning piglets, animals	10.33 ± 0.43^{a}	12.33 ± 0.44^{b}	11.21 ± 0.41^{ab}	11.33 ± 0.41^{ab}
Weight of litters of piglets at 28 days, kg	77.92 ± 3.45^{a}	106.73 ± 4.31^{b}	94.40 ± 3.03^{bc}	88.55 ± 3.95^{ac}
Average weight of piglet at 28 days, kg	7.533 ± 0.077^{a}	8.657 ± 0.163^{b}	8.443 ± 0.096^{b}	7.799 ± 0.124^{a}
Average daily gain from 0 to 28 days, g	223.9 ± 2.6^{a}	258.5 ± 4.6^{b}	250.7 ± 2.9^{b}	232.1 ± 4.1^{a}

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

In conditions of restricted feeding, sows with the AG genotype exceeded their counterparts with the GG genotype for piglets born alive per farrowing by 1.2 animals, or 10.0%, for weight of litters of piglets at birth – by 3.0 kg, or 20.1%, for the average weight of piglet at birth – by 0.1 kg, or 9.3%. According to indicators of productive traits established at weaning, the advantage of the AG genotype for average weight of piglet at 28 days was even greater – 0.6 kg, or 8.3% and for average daily gain from 0 to 28 days – 18.7 g, or 8.0%.

Conversely, in conditions of a high level of feeding, animals with the GG genotype had higher productivity in terms of such traits as piglets born alive per farrowing – by 1.6 animals, or 12.6%; weight of litters of piglets at birth – by 4.1 kg, or 22.4%; for average weight of piglet at birth – 0.2 kg, or 11.0%; by number of weaning piglets – 2.0 animals, or 16.2%; by weight of litters of piglets at 28 days – 28.8 kg, or 27.0%; for average weight of piglet at 28 days – 1.1 kg, or 13.0% and for average daily gain from 0 to 28 days – 34.6 g, or 13.4%. There was no significant difference between the groups that differed at the same time in terms of genotype and level of feeding ("AG + restricted level of feeding" and "GG + higher level of feeding" such as between groups "GG + restricted level of feeding" and "AG + higher level of feeding").

We researched the effect of sows' genotype and their feeding level during the growing, farrowing and weaning periods on the dynamics of live weight of piglets and their average daily gains at post-weaning period (Table 4).

Table 4
The significance of the influence of organized factors (feeding level and genotype of sows) on weight and growth of piglets after weaning

Productive traits	Feeding effect		Genotype effect		Interaction effect	
	F	p	F	p	F	p
Average weight of piglets at 60 days, kg	5.871	0.020	0.331	0.568	18.469	$4.28*10^{-5}$
Average daily gain from 28 to 60 days, g	0.454	0.504	0.000	0.990	0.415	0.523
Absolute gain from 28 to 60 days, kg	3.439	0.071	0.074	0.787	2.477	0.123
Relative gain from 28 to 60 days, %	0.069	0.795	2.670	0.110	13.853	0.001
Average weight of piglets at 90 days, kg	2.413	0.128	0.154	0.696	8.121	0.007
Average daily gain from 60 to 90 days, g	0.009	0.927	0.000	0.992	0.002	0.964
Absolute gain from 60 to 90 days, kg	0.009	0.927	0.000	0.992	0.002	0.964
Relative gain from 60 to 90 days, %	1.747	0.193	0.112	0.740	4.869	0.033

Table 5
Values of weight and growth in piglets obtained from sows with different genotypes and feeding levels during the farrowing and weaning periods (x ± SE)

Productive traits	High level feeding		Restricted feeding	
	AG (n = 15)	GG (n = 9)	AG (n = 14)	GG (n = 9)
Average weight of piglets at 60 days, kg	20.05 ± 0.33^{a}	22.03 ± 0.35^{b}	21.20 ± 0.19^{bc}	20.62 ± 0.22^{ac}
Average daily gain from 28 to 60 days, g	303.8 ± 14.8	303.1 ± 17.5	312.1 ± 16.6	290.3 ± 12.0
Absolute gain from 28 to 60 days, kg	12.52 ± 0.30	13.38 ± 0.28	12.76 ± 0.10	12.82 ± 0.22
Relative gain from 28 to 60 days, %	90.63 ± 1.12^{a}	87.18 ± 1.21^{ab}	86.10 ± 0.30^{b}	90.24 ± 1.26^{a}
Average weight of piglets at 90 days, kg	33.43 ± 0.37^{a}	35.37 ± 0.74^{b}	34.57 ± 0.33^{ab}	34.98 ± 0.29^{ab}
Average daily gain from 60 to 90 days, g	418.1 ± 9.5	416.7 ± 18.7	417.9 ± 4.6	417.4 ± 9.2
Absolute gain from 60 to 90 days, kg	13.38 ± 0.30	13.33 ± 0.60	13.37 ± 0.15	13.36 ± 0.29
Relative gain from 60 to 90 days, %	50.10 ± 1.17	46.36 ± 1.55	47.94 ± 0.14	48.93 ± 1.05

Note: see Table 3.

Only the live weight of their progeny at the age of 60 days was shown to be a valid indicator of the level of nutrition given to sows throughout the time of farrowing and nursing after the piglets had been weaned. The interaction of the mother's genotype with their feeding during growing and farrowing periods had a prolonged effect and affected the relative growth of their offspring in the post-weaning period, the average weight of piglets at the age of 60 and 90 days, and their relative growth from 60 to 90 days. After a month of rearing, the best live weight was characterized by the group of young pigs obtained from sows with the MC4R GG genotype and which received an increased ration during growth and lactation (Table 5).

The piglets from sows with the MC4R GG genotype which received an increased ration during growth and farrowing periods had a greater weight at 60 days from birth by 1.4 kg or 6.8% compared to the offspring of sows with the GG genotype (restricted feeding level) and by 2.0 kg or 9.9% more weight compared to the offspring of sows with the AG genotype (high level feeding).

As a result of determining the relative growth from 28 to 60 days, it was established that two groups (offspring of sows with genotype AG + high level of feeding and offspring of sows with genotype GG + restricted level of feeding) significantly prevailed over offspring of sows with genotype AG that received a restricted diet by 4.5 kg (or by 5.3%) and by 4.1 kg (or by 4.8%), respectively. At 90 days from birth young pigs obtained from sows with the GG genotype (the group receiving an increased ration) had a higher live weight by 2.0 kg or 5.8% compared to young pigs obtained from sows with the AG genotype and also receiving an increased ration. Thus, it was established that the live weight at the age of 60 and 90 days, as well as the relative growth of young pigs during rearing, is significantly influenced by the interaction of two organized factors (genotype of mothers MC4R AG / GG and the level of feeding of mothers during growth and lactation). A significant influence of these factors, taken separately, was found only for the level of feeding on live weight at the age of 60 days. That is, when evaluating animals by genotype, it is necessary to take into account not only the genetic factor, but also the interaction of the genotype with the environment.

The economic efficiency of the conducted research (Table 6) was determined on the basis of the difference in live weight and average daily gains for the period from birth to the end of rearing in young animals obtained from sows of different groups (with different genotypes and at different levels of feeding).

Table 6
Economic efficiency of growing piglets obtained from sows with different genotypes at different levels of feeding at Maxi 2010 LLC

Indicator	Group			
	high level feeding		restricted feeding	
	MC4R AG	MC4R GG	MC4R AG	MC4R GG
Number of piglets in the group, animals	80	80	80	80
Piglet rearing period from birth to transfer to fattening (weaning + rearing), days	90	90	90	90
The total number of piglets removed from rearing at 90 days, animals	80	80	80	80
Average daily growth for the entire growing period from birth to transfer to fattening, g	357.5	368.3	377.2	363.1
Gross production during the growing period, kg	2574.00	2651.76	2715.84	2614.32
Cost of kg of products, UAH	58.40	56.69	55.35	57.50
Total costs for the production of gross products, UAH	150322	150322	150322	150322
Purchase price of a product unit, UAH/kg	68	68	68	68
The cost of gross products at purchase prices, UAH	175032	180320	184677	177774
Net profit, UAH	24710	29998	34356	27452
Net profit per animal, UAH	309	375	429	343
The cost of additionally received products, UAH	–	5288	9645	2742
The level of profitability, %	16.4	20.0	22.9	18.3

Since all young animals were kept in similar conditions and received the same diet, the production costs were the same. The difference in productivity caused differences in the cost price, which in turn caused the difference in cost of gross products at purchase prices, net profit and the level of profitability.

The progeny of sows with the MC4R AG genotype given the restricted diet produced the highest net profit per head. The difference between groups MC4R AG/high level of feeding, MC4R GG/high level of feeding, and MC4R AG/restricted feeding was 121, 55 and 86 UAH, or 39.0%, 14.5%, and 25.1%, respectively.

Discussion

The Melanocortin 4 receptor gene is well known as a marker that has a substantial correlation with leanness, growth, and feed intake (Kim et al., 2000). Around 1800 animals from numerous commercial pig lines from the worldwide pig breeding corporation PIC were investigated for the association of MC4R genotypes with the impacts on variance in growth rate, backfat, and feed intake in order to investigate the implications of the missense MC4R gene mutation. For all performance traits, a significant association with the MC4R genotypes was discovered. When compared to the homozygous AA genotype animals, the animals homozygous for allele G had on average considerably less backfat ($P < 0.001$), poorer daily gain ($P < 0.001$), and reduced feed intake ($P < 0.01$). Pigs with the AA genotype grew substantially quicker (37 g/day) than pigs with the GG genotype, although overall, pigs with the GG genotype had about 9% less backfat than pigs with the genotype. The AA genotype animals take significantly more feed, hence these findings seem to be a function of appetite. However, in studies by Calta et al. (2022) according to the general linear model, MC4R had no statistically significant overall effect, and the realization of genetic potential depends on the level of animal feeding. Although the overall impact of polymorphism on the sample they chose was minimal, there were a few notable disparities between the two dietary categories. Allele G encouraged leanness while allele A encouraged more gain in the high level category. Contrarily, the results from the category of restricted feeding indicated that food might change the effects of both alleles. Those results suggest that the MC4R polymorphism can be utilised for selection purposes in light of earlier studies. These results need to be clarified by additional research, such as gene expression in various feeding techniques.

On the other hand, it is known that obesity of pigs leads to metabolic disease in late pregnant sows and has been linked to decreased litter weight, constipation at farrowing, and detrimental impacts on the intestinal health of developing piglets (Cheng et al., 2020; Peltoniemi et al., 2023). However, according to other scientists (Prunier & Quesnel, 2000; Waller et al., 2006) a positive energy balance, together with excellent body condition and a high body condition score, are thought to be advantageous for proper cyclicity and fertility in a number of species, including pigs. In terms of sow cyclicity, severe body condition loss during lactation may be more of a problem than overconditioning (Prunier & Quesnel, 2000; Han et al., 2020). But nevertheless pigs that are overfed or underfed for all or part of the reproductive cycle are more likely to experience metabolic problems and have poor reproductive health, which reduces sow lifespan and raises perinatal mortality. This causes poor animal welfare in addition to being an economic issue for the pig business (Muro et al., 2022). Since previous studies (Vashchenko et al., 2023) found that the MC4R genotype, under the condition of a high level of feeding, leads to an increase in the thickness of the backfat in pigs by 8.1–11.3%, therefore, in this work, the emphasis was placed on finding the effect of this marker on reproductive ability.

The optimal value of Polymorphism Information Content (PIC) for conducting associative studies is between 0.25 and 0.75 units (Botstein et al., 1980). The value of PIC established in our studies was equal 0.35, which gives the required variety of genotypes to demonstrate their correlations with productivity indicators. Indeed, sows from the "MC4R AG + high level feeding" group had worse fertility compared to the "MC4R GG

+ high level feeding" group: number of piglets born alive was less by 1.6 head (14.1%; P = 0.023), weight of litters of piglets at birth was less by 4.1 kg (28.9%; $P = 2.21*10^{-5}$) and average weight of a piglet at birth was less by 0.16 kg (12.7%; P = 0.003). However, when feeding of animals with the AG genotype was restricted, there was no significant difference between them and "MC4R GG + high level feeding" animals. Thus, to achieve the best results in terms of fertility, it is necessary to take into account the tendency of sows with the A allele to obesity and reduced reproductive capacity and feed them a limited diet. As for animals with the GG genotype, the restriction of their daily ration caused deterioration in fertility, that is, to obtain optimal results, it is necessary to provide them with an increased level of feeding. In general, these results are consistent with the well-known facts that animals of breeds with better meatiness (Pietren, Durok) have a worse reproductive capacity compared to breeds of the "maternal" productivity type, such as Large White and Landrace (Zhang et al., 2020; Alam et al., 2021; Ganteil et al., 2021).

The effect of the level of feeding was established for the traits measured after the end of the suckling period. This is explained by the fact that the amount of milk produced by lactating sows directly depends on the supply of nutrients. The results obtained by us on the influence of feeding on the growth of suckling piglets are consistent with the work of the Tan et al. (2018).

It should be noted that after weaning a significant separate influence of genotype was established only for the live weight of piglets at the age of 28 days, which is explained by the different nature of the influence of genotype under different feeding conditions. Our results are consistent with the opinion of other researchers (Calta et al., 2022), who found in studies that hybrid MC4R AG animals use the diet more efficiently under conditions of limited feeding. This is explained by the fact that, animals with the A allele tend to consume more feed, which is important for ensuring a sufficient amount of nutrients for the normal process of offspring formation and piglet feeding. Simultaneously with this, the G allele of hybrid pigs is associated with a less intensive growth of fat and a greater intensity of growth of muscle tissue, which requires half as much feed for the formation of a similar amount of live mass, compared to adipose tissue (Vashchenko et al., 2019). Thus, in conditions of limited feeding, animals with the G allele use feed more efficiently, and the presence of the A allele contributes to greater feed consumption.

Pigs that received a high level of ration at an early age and had the GG genotype when they reached reproductive maturity were distinguished by better values of the number piglets born alive per farrowing (+12.6%), better values of weight of litters of piglets at birth (+22.4%), better average weight of one piglet at birth (+11.0%), number of weaning piglets (+16.2%), better values of weight of litters of piglets at 28 days (+27.0%), average weight of one piglet at 28 days (+13.0%) and better average daily gain from 0 to 28 days (+13.4%). These results are consistent among a significant number of researchers (Nyachoti et al., 2004; Elbert et al., 2020; Faccin et al., 2020), who also noted the intensity of growth of piglets in the post-weaning period affects their further growth, development and reproductive capacity.

After the weaning of piglets, a reliable effect of the level of feeding of sows during the period of farrowing and lactation was established only on the live weight of their offspring at the age of 60 days. That is, in this study, no prolonged effect of better feeding of sows on their offspring was observed. This may be due to the insufficiently large numbers of animals in our study. The influence of the mother's genotype at this stage was not detected at all. Most cases of reliable influence were found for the interaction of organized factors. A particularly "sensitive" trait to the influence of the interaction of the genotype and the feeding of the sow-mothers of the experimental young animals was the relative growth – a significant difference was recorded in both months of rearing.

The revealed influence of the interaction of the MC4R genotype and the level of feeding caused significant differences in economic results from obtaining farrowings of experimental sows. The level of profitability in groups with the optimal combination of genotype and feeding factors ("MC4R GG + high feeding level" and "MC4R AG + restricted feeding level") was 1.7–6.5 percentage points better compared to the other two groups. These results confirm the importance of the reproductive capacity of sows for the economic efficiency of pork production (Chen et al., 2019; Zhang et al., 2019; Van Hung et al., 2022).

Conclusions

It was established that the traits of reproductive ability (piglets born alive per farrowing, weight of litters of piglets at birth, average weight of piglet at birth, number of weaning piglets, weight of litters of piglets at 28 days, average weight of piglet at 28 days, average daily gain of piglets from 0 to 28 days) of crossbred Large White × Landrace pigs was affected by the interaction of genotype and feeding factors. When limiting the nutritional value of the daily diet, sows with the AG genotype outperformed those with the GG genotype by 1.2 piglets born alive per farrowing, or 10.0%, for litter weight at birth, by 3.0 kg, or 20.1%, and for the average weight of a piglet at birth, by 0.1 kg, or 9.3%. The advantage of the AG genotype for average piglet weight at 28 days was even larger – 0.6 kg, or 8.3% – and for average daily growth from 0 to 28 days – 18.7 g, or 8.0% – according to indices of productive features determined at weaning. Conversely, in conditions of a high level of feeding, animals with the GG genotype had higher productivity in terms of such traits as piglets born alive per farrowing – by 1.6 animals, or 12.6%; weight of litters of piglets at birth – by 4.1 kg, or 22.4%; for average weight of piglet at birth – 0.2 kg, or 11.0%; by number of weaning piglets – 2.0 animals, or 16.2%; by weight of litters of piglets at 28 days – 28.8 kg, or 27.0%; for average weight of piglet at 28 days – 1.1 kg, or 13.0% and for average daily gain from 0 to 28 days – 34.6 g, or 13.4%.

After piglets were weaned, the effect of the interaction of organized factors continued to affect such characteristics as absolute and average daily gain from 60 to 90 days. At 90 days from birth, young pigs obtained from sows with the GG genotype (the group receiving an increased ration) had a higher live weight by 2.0 kg or 5.8% compared to young pigs obtained from sows with the AG genotype and also receiving an increased ration.

The MC4R AG genotype sows' offspring that were fed a limited diet had the highest net profit per head. Groups "MC4R AG/high level of feeding" had lower net profit at 39.0%, 25.1% and 14.5% compared to "MC4R AG/restricted feeding", "MC4R GG/high level of feeding" and "MC4R GG/restricted feeding" respectively.

This study was carried out with the support of the National Academy of Agrarian Sciences of Ukraine at the expense of the budget project "Innovative technologies of breeding, industrial and organic production of pig products." The subproject was "To investigate the peculiarities of the formation of high-performance genotypes of pigs and their adaptive abilities in the conditions of climate change", state registration No. 0121U109839.

The authors declare no conflicts of interest.

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