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ASSOCIATION OF MITOCHONDRIAL HAPLOTYPES WITH PHENOTYPIC SIGNS OF FATTENING PRODUCTIVITY IN HYBRID PIGS

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The results of the study indicate that the variability of mtDNA of pigs is one of the ways to assess and predict the productivity of hybrid pigs by phenotypic characteristics. The purpose of the study was to conduct zootechnical analysis to determine the presence of an association of mtDNA haplotypes with signs of fattening productivity in hybrid pigs (Large White × Landrace). The study was conducted on an experimental sample of pigs (n = 18), which are the result of direct (Large White × Landrace) and reciprocal crossing (Landrace × Large White). DNA extraction was performed from epithelial tissue using a kit DNA-sorb-B nucleic acid extraction kit from "InterLabService-Ukraine" LLC. A previous study identified 4 mtDNA haplotypes among hybrid pigs of Irish selection, three of which are grouped with pigs of European origin clade "E" – C (Landrace, Hampshire, Wales, wild pig); G (Wales, wild pig); O (Landrace, wild pig). Pigs with haplotype N (Large White, Berkshire, Asian wild pig) grouped with pigs of Asian origin. Asian-type pigs with mitochondrial haplotype N belong to the Asian cluster «clade A». The common origin of hybrid pigs has been determined and it has been clarified and how these mitochondrial haplotypes affect fattening productivity has been identified. The estimated main phenotypic indicators are the following average values for relation to mitochondrial haplotypes in pigs. (C, G, O, N): a) average daily weight gain for the fattening period, kg/g – haplotype C (867 g), N (835 g) 77%; for haplotype G (761 g) 74% and 75% (789 g) for haplotype O. b) the time spent in the fattening group, days, is 10% (107 days) for all haplotypes (C, G, O, N). c) Wet weight attainment, 100 kg/day, for haplotype C (144 days), N (141 days) 13 percent; 16% (165 days) for haplotype G and 15% (156 days) for haplotype O. Transfer to the rearing group was carried out at the same age of 63 days, but with a fairly large difference in weight of 5 and 9.2 kg, after all, the average value when fattening should be 30 kg. This also indicates an uneven growth of the studied sample of hybrid pigs. This is clearly reflected throughout the life of the studied pigs in the following indicators: the age of achievement of live weight is 100 kg/ day for the accounting period from birth before slaughter, for haplotype C – 144 and N – 141 days compared to pigs with haplotype O – 156 and G – 165 days with a difference of 13 and 22 days. The age of achievement of live weight is 100 kg/day for the accounting period from days of fattening before slaughter, for haplotype C – 150 and N – 145 days compared to pigs with haplotype O – 159 and G – 166 days with a difference of 11 and 18 days. Pigs with haplotypes C and N are characterized by a uniform average daily gain over the fattening period with an average of 851 g with a difference of -76 g with haplotypes O and G. It is worth noting that only all pigs with haplotypes C (0.696 g), G (0.605 g), O (0.642 g), N (0.715 g) – are characterized by a uniformly low average daily gain for the entire period of cultivation (from birth to removal from fattening). A logical assumption is that there are advantages of life priority for born pigs, who have an adequate birth weight to be profitable. These facts confirm the prospect of continuing research on the association of mtDNA haplotypes as determinantspig of productivity.

Key words: *pigs, haplotype, haplogroup, mitochondrial genome, D-loop, mtDNA haplotype association, clade, cluster, PCR-RFLP analysis, zootechnical analysis, the gender difference in pigs, AGE100, ADG.*

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The search for cause-and-effect relationships between genotype and phenotype is an urgent problem in the mitochondrial biology of highly productive animals (Fabrizio Ghiselli, 2019; Milani L, 2019). After all, we do not know how dynamics (division and fusion of mitochondrial networks) affects the distribution of mtDNA variants and how they affect the animal's phenotype. To provide a true assessment of the productive characteristics of pigs, the study of the variability of the mtDNA of pigs is one of the ways to assess and predict breeding, commodity, and productive traits. The accuracy of the conditional calculation of the herd management strategy to obtain the desired phenotypic characteristics is recommended to register fattening and other indicators of pigs (Madonna Benjamin, 2019; Mareike Pfeifer, 2020; Luiz F. Brito, 2020). Mitochondrial DNA is a determining factor in the phenotypic traits of hybrid pigs. The mitochondrial genome is inherited strictly along the maternal line of the population of mitochondria present in the egg during fertilization (Jeffrey H. Schwartz, 2021). Due to the fact that the mitochondrial genome plays an important role in the production of energy and the control of cell functions, this significantly affects the traits of farm animals that are significant for breeding in general (Guanghui Yu., 2015; J. Häggman, 2016). With this in mind, it became the goal of conducting a study in determining the association of mtDNA haplotypes with phenotypic traits in hybrid pigs. The maternally inherited mitochondrial genome is double-stranded. In a pig, the mitochondrial genome has approximately 16,700 base pairs (Björn M. Ursing, 1998; Te-Sha Tsai, 2016). The maternally inherited mitochondrial genome is required for the biochemical process of oxidative phosphorylation (OXPHOS), which generates most of the cellular energy (ATP) (Te-Sha Tsai, 2016; Thomas Pfeiffer, 2001). OXPHOS is carried out in the electron transport chain and is the only cellular apparatus, subunits of which are encoded by chromosomal and mitochondrial genomes (Te-Sha Tsai, 2016; Anderson S., 1981). The mitochondrial genome encodes 13 subunits of the electron transport chain, 22 tRNAs and 2 rRNAs (Hieu Duc Nguyen, 2017; Anjana Saravanan, 2022). The mitochondrial genome has one major non-coding region – the D-loop, which is where the transcription and replication factors encoded by the kernel interact, which travel to the mitochondria to initiate transcription, and then replication of the mitochondrial genome. The D-loop also has two hypervariable regions (HVI and HVII), which are used to identify maternal hereditary models of mtDNA transmission and migration patterns of wild and domesticated pigs worldwide (Eduardo Ruiz-Pesini, 2004; Te-Sha Tsai, 2016). There are a number of traits important to the production of pigs, which are collectively defined as estimated marketable value. Estimated commercial value allows you to assess the overall productivity of the studied sample of animals. The results obtained are important information for the breeding, commodity and meat industries. Key criteria include reproductive factors such as number of live births, size droppings and quality of nipples; factors related to meat quality, such as fat density and muscle thickness; average daily gain during life (Yen N. T., 2007) namely the assessment of weight gain between birth and death, the rate of growth (Kanis E., 2005). These criteria will determine whether it is possible to maintain farm animal lines in breeding programs and how they will breeding programs are managed. As citizens and consumers increasingly attach importance to characteristics, which have little or no direct relation to the cost or price of the product, pig-breeding organizations want to pay more attention to socially important characteristics, such as the welfare and health of pigs, the environmental impact of pork production, as well as the usefulness and organoleptic qualities of the pork product (Kanis E., 2005). Since mtDNA haplotypes are associated with adaptation to the environment, diseases, and reproductive functions (Justin C. St. John, 2018), we decided to determine, if there are other specific performance traits associated with pigs that indicate their estimated commercial value, depend on their mtDNA haplotypes, and in particular, whether these haplotypes will be associated with fattening productivity, to promote other specific phenotypic and genotypic traits, this became the interest of our research.

The purpose of the study. Conduct a zootechnical analysis to determine the presence of an association of mtDNA haplotypes with phenotypic signs of fattening performance of hybrid pigs.

Materials and methods of research. Data obtained from pigs (n=18) Irish selection. All pigs were raised in TOV NVP "Globinsky pig farm", Poltava region, Ukraine. The experimental sample of pigs under study are direct descendants in the maternal line according to the results of the previous study, which have been assigned to mtDNA haplotypes from C to N (Budakva Ye.O.,2022; Pochernyaev K.F., 2014; Pochernyaev K.F. 2005)]. For the study, we selected biological material – pig ears during the slaughter of animals at the «Globino» meat processing plant. DNA extraction was performed from epithelial tissue using the DNA-sorb-B nucleic acid extraction kit from "InterLabService-Ukraine" LLC (Budakva Ye.O., 2022). The mitochondrial haplotypes were determined using the PCR-RFLP method. DNA amplification by PCR was performed using recombinant Taq DNA Polymerase (Thermo Scientific™), according to the manufacturer's recommendations. Amplification was carried out on the programmable thermostat TERTSYK-2 (DNA-Technologies) under conditions of synthesis, depending on the structure of oligonucleotide primers: forward – MITPRO2F *CATACAAATATGTGACCCCAAA,* and reverse – MITPROR *GTGAGCATGGGCTGATTAGTC*. Specificity of PCR products was tested using 2% agarous gel. Hydrolysis of PCR products using TasI endonuclease restriction (↓AATT) was performed in accordance with the manufacturer's instructions (Thermo Scientific™). DNA hydrolysis products were analyzed in 8% polyacrylamide gel in electrophoresis buffer 1×TBE. As a marker of molecular weight, pBR322 DNA/MspI plasmid DNA was used, and pUC19 DNA/MspI. Visualization of amplification and destruction products was carried out by painting with bromide ethidium and photographing on the transilluminator in ultraviolet light (MicroDOC Gel Documentation Digital camera with UV Transilluminator, Cleaver Scientific).

Results. To evaluate the main phenotypic traits in the studied sample of pigs (Large White × Landrace) × Maxgro, we calculated the indicators of fattening productivity, which are presented in (Table 1, Figure 1, 2).

From (Table 1 and Diagram 1) in relation to the C, G, O, N haplotypes: The average age at transfer to the growing group is 19.5 days; time spent in the growing group – 44 days; age (days) when transferring to the fattening group – 63; time spent in the fattening group – 107 days; age (days) of removal from fattening – 171. The difference is in the following indicators: in weight when fattening, kg/day – pigs with haplotype G (22 kg) by 5 kg compared to haplotypes C and O (27 kg) previously, fattening was carried out and with a difference of 9.2 kg compared to haplotype N. The results show that the transfer to the rearing group was carried out at the same age of 63 days, but with a fairly large difference in weight of 5 and 9.2 kg, after all, the average value when fattening should be 30 kg. This is also indicative of the uneven growth of the study sample of hybrid pigs. This is clearly reflected throughout the life of the pigs under study according to the following indicators: the age of achievement of live weight is 100 kg/ day for the accounting period frombirth before slaughter, for haplotype C – 144 and N – 141 days compared to pigs with haplotype O – 156 and G – 165 days with a difference of 13 and 22 days. The age of achievement of live weight is 100 kg/day for the accounting period from days of fattening before slaughter, for haplotype C – 150 and N – 145 days compared to pigs with haplotype O – 159 and G – 166 days with a difference of 11 and 18 days. Pigs with haplotypes C and N are characterized by a uniform average daily gain for the fattening period with an average of 0.851 g with a difference of +76 g with haplotypes O and G – 0.775 g. It is worth noting that all pigs with haplotypes C (0.696 g), G (0.605 g), O (0.642 g), N (0.715 g) – are characterized by a uniformly low average daily gain for the entire period of cultivation (from birth to removal from fattening).

Graphs (Figure 2) represent the average values of the main phenotypic features relative to the mtDNA haplotype pigs (*C, G, O, N*): a) average daily weight gain for the fattening period, kg/g – haplotype C, N (n=6) 77%; for haplotype G (n=1) 74% and 75% for haplotype O. b) the time of stay in the fattening group, days, is 10% (44 days) for all

Table 1

Evaluation of phenotypic traits relative to the mtDNA haplotype in the hybrid pig population under study (Large White × Landrace) × Maxgro

Evaluated indicators:	Haplotypes			
	C (n = 6)	O (n = 5)	N (n = 6)	G (n = 1)
Age (days) when transferred to the growing group	20	19	20	19
Time spent in the growing group, days	44	44	44	44
Age (days) when transferred to the fattening group	64	63	64	63
Weight when setting for fattening, kg	27	27	31,2	22
Weight when removed from fattening, kg	119	109	123	103
Weight gained during fattening period, kg	93	83	92	82
ADG (kg/g) during fattening period	867	789	835	761
Relative growth,%	1,276	1,202	1,198	1,301
Time spent in the fattening group, days	107	107	107	107
Age (days) of fattening	171	170	171	170
ADG for the entire period of cultivation, kg/g	696	642	715,3	605
Age (days) reaching a live weight of 100 kg for the accounting period from birth to slaughter	144	156	141	165
Age (days) reaching a live weight of 100 kg for the accounting period from the day of fattening to slaughter	150	159	145	166

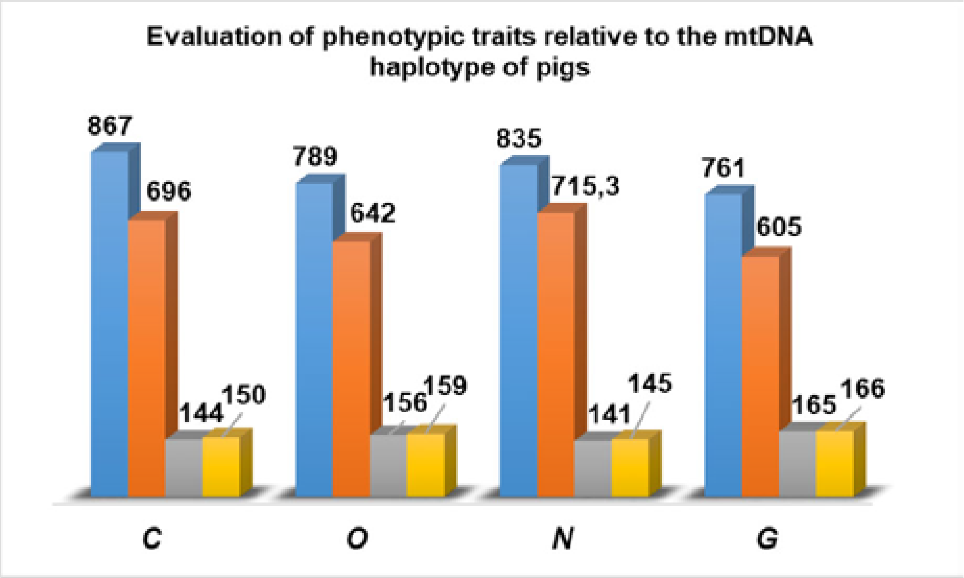

Figure 1. Phenotypic trait evaluation diagram relative to the mtDNA haplotype of hybrid pigs (Large White × Landrace) × Maxgro (n=18)

haplotypes (*C, G, O, N*). c) age of reaching live weight, 100 kg/day, for haplotype C, N (n=6) 13%; 16% for haplotype G, and 15% for haplotype O. Representatives (Sus scrofa) are assigned mtDNA haplotypes, a characteristic feature of haplotypes is a close grouping of sequence – haplogroup. Thus, the studied population has a common origin, and their migration patterns can be mapped.

Discussion. Moreover, there seems to be a compromise between productive ability and other signs such as AGE100 and ADG, what is demonstrated by mitochondrial haplotype C and N. While this is not unusual, this highlights why certain animals are supported in breeding programs, because they have signs that provide profitability, but at the expense of other characteristics. Evaluation of mtDNA haplotypes in hybrid pigs (Large White × Landrace) × Maxgro and using this additional information to change breeding strategies can significantly increase the estimated tribal value, thus, to ensure further improvement of certain populations of transboundary and local pig breeds. Genetic monitoring of young pigs of GGP/GP breeds of large white, landrace on the maternal line and terminal line Maxgro from Ireland and Ukraine in breeding programs Hermitage BreedDirect BLUP is focused on individual customer requirements. However, monitoring the evaluation of the association for X and Y-chromosomal haplotypes of the broodstock and paternal herd on the signs of target productivity in conjunction with intra-breed QTL DNA-markers – is an actual addition to

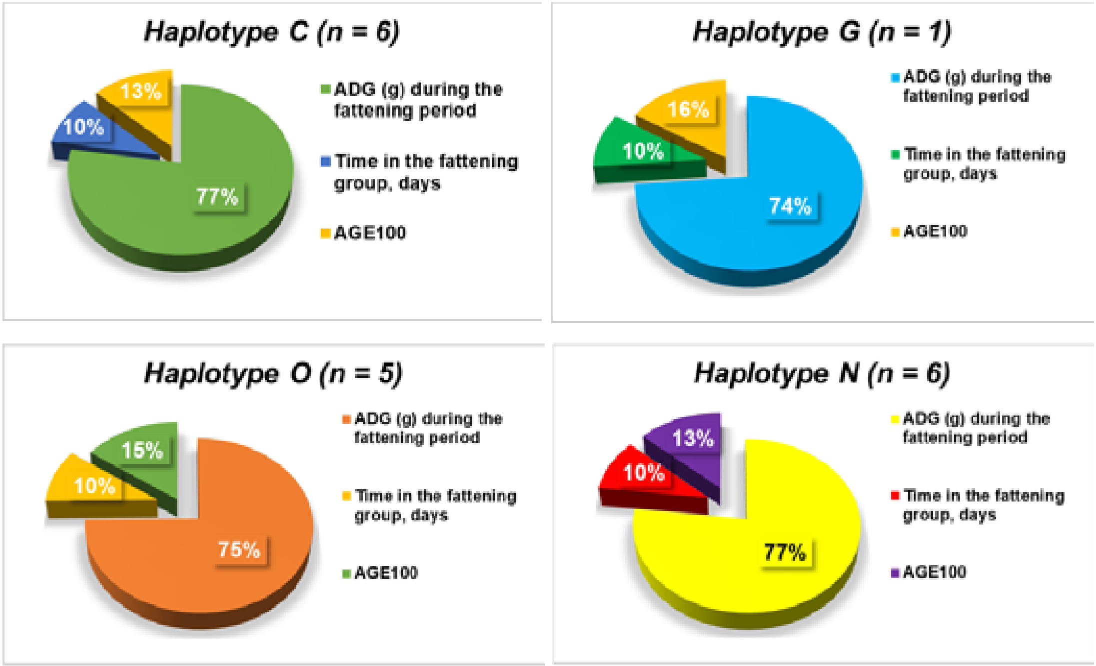

Figure 2. The average value of the main phenotypic traits relative to the mtDNA haplotype in hybrid pigs (Large White × Landrace) × Maxgro (n=18)

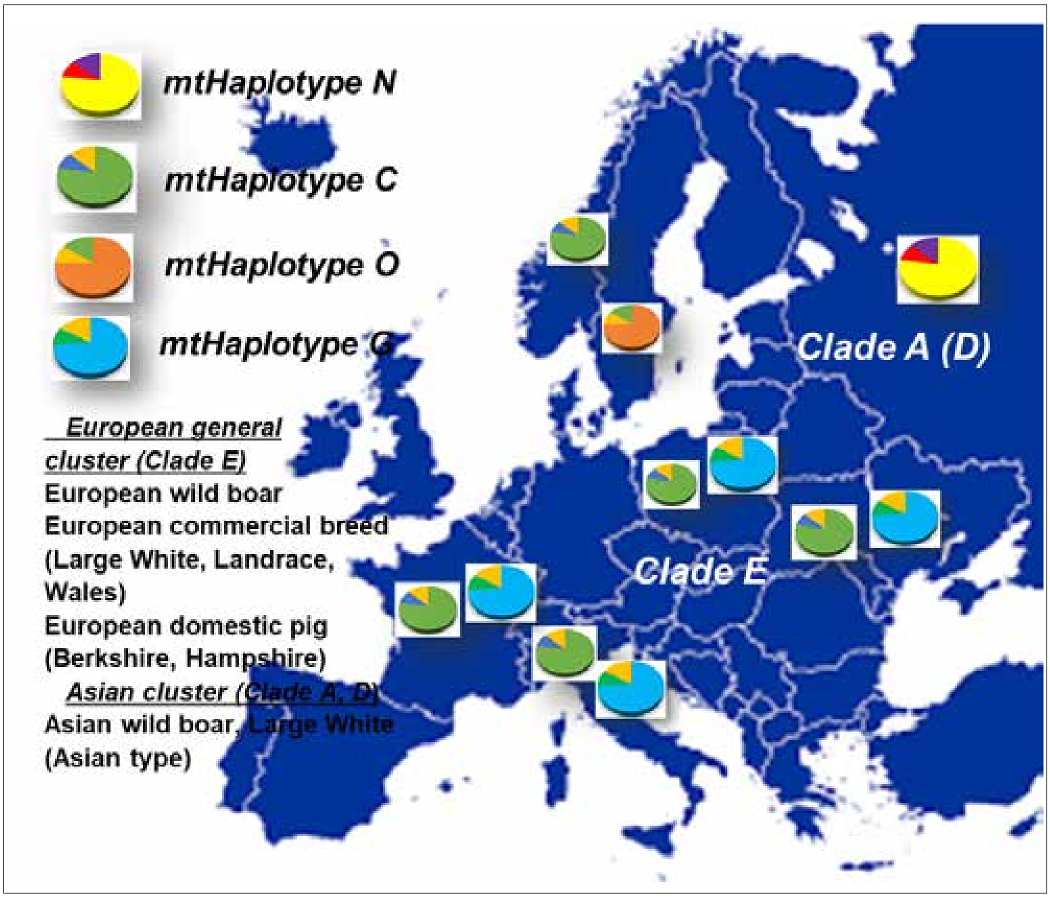

Figure 3. Clustering of mtDNA haplotypes from the D-loop area of 18 commercially pigs. Map showing Southeast Asia, place of origin of pig species (Sus scrofa), as well as the current geographical distribution of wild and domesticated pigs. The clade A: N – Large White (Asian type), Berkshire, Asian wild pig. The clade E: C – Landrace, Hampshire, Wales, wild pig (Ukraine, Poland, France); O – Landrace, wild pig (Sweden, France); G – Wales, wild pig (Italy)

breeding programs. Our data show that mtDNA haplotypes are associated with a number of important phenotypic traits, indicating the economic value of hybrid pigs with "gender" differences. We see to continue the definition of associations of mtDNA haplotypes in the studied population of hybrid pigs with economically useful features.

Conclusions. Overall, the study shows that mtDNA haplotypes confer positive benefits on phenotypes such as growth rate during fattening to slaughter, such as growth rate during fattening to slaughter – for pigs of Asian type (haplotype N) and European type (haplotype C and O). Evaluation of phenotypic traits determines the effectiveness of breeding work and the economic potential of the studied population of hybrid pigs (Large White × Landrace).

Prospects for further research. Due to the fact that mtDNA haplotypes are associated with important phenotypic signs, indicating the economic value of hybrid pigs with «gender» differences. We see to continue the definition of associations of mtDNA haplotypes in the studied population of hybrid pigs with economically useful features. Conduct an associational analysis of QTL markers MC4R (c.1426 A>G), LEPR (g.2856 C>T), CTSD (g.70 G>A), RYR1 (g.1843 C>T), IGF-2 (g.3072G>A) with mitochondrial DNA markers to assess the phenotypic signs of pig productivity.

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Асоціація мітохондріальних гаплотипів з фенотиповими ознаками відгодівельної продуктивності гібридних свиней

Результати проведеного дослідження свідчать про те, що мінливість мтДНК свиней є одним із способів оцінки та прогнозування продуктивності гібридних свиней за фенотиповими ознаками. Метою дослідження було проведення зоотехнічного аналізу для визначення наявності асоціації гаплотипів мтДНК з ознаками відгодівльної продуктивності у гібридних свиней (велика біла × ландрас). Дослідження проведено на експериментальній вибірці свиней (n = 18), котрі є результатом прямого (велика біла × ландрас) та реципрокного схрещування (ландрас× велика біла). Екстракція ДНК проведена з епітеліальної тканини з використанням набору DNA-sorb-B nucleic acid extraction kit від ТОВ «ІнтерЛабСервіс-Україна». У попередньому дослідженні виявлено 4 гаплотипи мтДНК серед гібридних свиней ірландської селекції, три з яких згруповані зі свинями європейського походження «клада Е» – представники гаплотипу C (ландрас, гемпшир, велика біла, дика свиня); G (уельс, дика свиня); O (ландрас, дика свиня). Свині з гаплотипом N (велика біла, беркшир, азіатська дика свиня) згруповані зі свинями азіатського походження. Свині азіатського типу з мітохондріальним гаплотипом N належать до азіатського кластера «клада А». Визначено спільне походження гібридних свиней та з'ясовано, як ці мітохондріальні гаплотипи впливають на відгодівельну продуктивність. Передбачувані основні фенотипові показники являють собою наступні середні значення по відношеню до мітохондріальних гаплотипів у свиней (C, G, O, N): a) середньодобовий приріст за період відгодівлі, кг/г – гаплотип C (867 g), N (835 g) 77%; для гаплотипа G (761 г) 74% і 75% (789 г) для гаплотипа O. b) час перебування в групі відгодівлі (днів), становить 10% (107 днів) для всіх гаплотипів (C, G, O, N). c) вік досягнення живої маси 100 кг/дн., для гаплотипа C (144 дн.), N (141 дн.) 13%; 16% (165 дн.) для гаплотипа G і 15% (156 дн.) для гаплотипа O. Переведення в групу дорощування проводили в тому ж віці 63 дні, але з досить великою різницею у вазі 5 і 9,2 кг, адже середнє значення при постановці на відгодівлю повинно бути 30 кг. Це також свідчить про нерівномірність росту досліджуваної вибірки гібридних свиней. Це чітко відображається протягом життя досліджуваних свиней у наступних показниках: вік досягнення живої маси 100 кг/днів за обліковий період від народження до забою, для гаплотипу C – 144 і N – 141 днів порівняно зі свинями з гаплотипом O – 156 і G – 165 днів з різницею у 13 і 22 дні. Вік досягнення живої маси 100 кг/днів за обліковий період від дня відгодівлі до забою, для гаплотипу C – 150 та N – 145 днів порівняно зі свинями з гаплотипом O – 159 та G – 166 днів з різницею в 11 і 18 днів. Свині з гаплотипами C і N характеризуються рівномірним середньодобовим приростом за період відгодівлі в середньому 851 г з різницею -76 г з гаплотипами O і G. Варто зазначити, що свині з гаплотипами C (0,696 г), G (0,605 г), O (0,642 г), N (0,715 г) – характеризуються рівномірно низьким середньодобовим приростом за весь період вирощування (від народження до зняття з відгодівлі). Логічним припущенням є те, що існують переваги життєвого пріоритету для народжених свиней, які мають достатню вагу при народженні, щоб бути прибутковими. Ці факти підтверджують перспективність продовження досліджень асоціації гаплотипів мтДНК як детермінант продуктивності.

Ключові слова: *свині, гаплотип, мітохондріальний геном, D-петля, асоціація гаплотипу мітохондріальної ДНК, клада, кластер, ПЛР-ПДРФ, зоотехнічний аналіз, AGE100, ADG.*