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TRANSFORMING GROWTH FACTOR B1, PITUITARY-SPECIFIC TRANSCRIPTIONAL FACTOR 1 AND INSULIN-LIKE GROWTH FACTOR I GENE POLYMORPHISMS IN THE POPULATION OF THE POLTAVA CLAY CHICKEN BREED: ASSOCIATION WITH PRODUCTIVE TRAITS

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Aim. To investigate the gene polymorphisms of transforming growth factor β1 (TGF-β1), pituitary-specific transcriptional factor 1 (PIT-1) and insulin-like growth factor I (IGF-I) in the population of Poltava clay chicken breed, used for egg and meat production, and to analyze the association of different genotypes for each locus with productive traits. **Methods.** Genotyping of the chickens was performed using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). **Results.** Transforming growth factor TGF-β1, pituitary-specific transcriptional factor PIT-1, and insulin-like growth factor IGF-I were shown to be polymorphic in the studied populations. The association between genotypes by the loci TGF-β1 and PIT-1 and the indices of egg and meat production of chickens was demonstrated. **Conclusions.** The data on the genetic structure of the population of Poltava clay chicken breed by loci TGF-β1 and PIT-1 is recommended for the targeted selection of chickens to produce offspring with desirable genotypes, which will, in addition to classical breeding methods, reveal the productive potential of chickens as efficiently as possible.

Keywords: Poltava clay chicken breed, polymorphism, transforming growth factor β1, pituitary-specific transcriptional factor 1, insulin-like growth factor I, productive traits.

INTRODUCTION

Successful development of poultry industry is considerably determined by the efficiency of selection. Due to recent rapid development of molecular and genetic methods of research there is a new possibility of putting selection into practice on the essentially new foundation – applying the data on polymorphism of target genes and their association with productivity traits. This approach, called marker-assisted selection (MAS-selection), is based on the study of polymorphism of the selected genes, the association of allele variants with productive traits, the categorization of individuals according to the complex of genes, and the selection of individuals with desirable genotypes for further breeding [1]. An important stage of research in this context is the selection of target genes for the study of their polymorphism in experimental populations. The investigation of polymorphism of candidate genes in the studied populations of chickens is the first step, required for further analysis of the association of different genotypes with productive traits which may be directly used in the selection [2]. The promising candidate genes, the association of which to poultry productivity traits was proven in numerous foreign investigations, include, in particular, such objects as the genes of transforming growth factor β1 (TGF-β1), pituitary transcriptional factor 1 (PIT-1) and insulin-like growth factor I (IGF-I).

TGF-β1 belongs to one of the most important groups of genes (family of transforming growth factors β), participating in the maintenance of tissue homeostasis, growth and differentiation of different types of cells, the forma-

tion of intercellular matrix, the induction of apoptosis, the regulation of the immune system, etc [3]. Gene TGF-β1 is localized on the 13th chromosome and contains 17 exons. It was demonstrated that allele variants of TGF-β1 correlate with meat production traits of chickens of different breeds [4].

PIT-1 participates in the expression regulation of growth hormone genes, prolactin, thyreotropin, proliferation and differentiation of hormone-secreting cells, the control of immune system development [5]. Its functioning is directly related to that of the controlled genes (growth hormone, prolactin) which defines PIT-1 as a promising candidate for the study of the association of different genotypes and the productivity traits due to the especially important role of prolactin in the regulation of the reproductive cycle of birds. PIT-1 gene contains 6 exons and 5 introns. Several SNP were revealed in different gene regions [5]. It was shown that allele variants of PIT-1 gene are associated with the live weight of chickens of different breeds [6].

IGF-I performs a number of physiological functions, related to the growth and differentiation of tissues, and belongs to the family of insulin-like growth factors [7]. Its functioning is closely related to the growth hormone and it is an active participant of the growth regulation and development of the embryo. IGF-I gene contains 4 exons and 3 introns. High level of polymorphism was registered for 5′ and 3′ noncoding regions (5′UTR and 3′UTR), as well as intron and exon parts of IGF-I gene. The association of different allele variants of the gene and productive traits of chickens was studied [8−10].

Previously we investigated the genetic structure of the populations of chickens, bred in Ukraine for egg and meat production by loci of prolactin, growth hormone, growth hormone receptor, family of transforming growth beta-factors, etc [11, 12].

The current work was aimed at the analysis of the polymorphism of genes TGF-β1, PIT-1 and IGF-I in the population of Poltava clay chicken breed, bred for egg and meat production, as well as the determination of the association of different genotypes by each of the loci with productivity traits.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Poultry Disease Prevention and Molecular Diagnostics of the State Poultry Experimental Station NAAS of Ukraine, using the Poltava clay chicken breed, line 14 (n = 100), selected in Ukraine for egg and meat production. The biological material (blood) was sampled from each chicken at the experimental poultry farm "Preservation of State Genetic Pool of Poultry" of the State Poultry Experimental Station NAAS of Ukraine. The blood sample was taken from the crown using the scarificator and sterile filter paper. Each sample was dried, marked and individually packed to prevent any contamination. DNA was isolated from the experimental samples using the commercial kit of reagents DNA-sorb-B (AmpliSens, RF). The efficiency of DNA isolation was determined by electrophoresis in 0.7 % agarose gel at 200 V for 5 min.

The following parameters were used for the amplification:

TGF-β1 – Forward: 5′-GGGGTCTTCAAGCTGAGCGT-3′, Reverse: 5′-TTGGCAATGCTCTGCATGTC-3′ [4];

PIT-1 – Forward: 5′-GTCAAGGCAAATATTCTGTACC-3′, Reverse: 5′-TGCATGTTAATTTGGCTCTG-3′ [5];

IGF-I – Forward: 5′-GACTATACAGAAAGAACCAC-3′, Reverse: 5′-TATCACTCAAGTGGCTCAAGT-3′ [9].

The amplification was conducted using the reagents of DreamTaq PCR Master Mix (ThermoScientific, USA) and programmed thermocycler Tertsik (DNA-technologies, RF) using the corresponding programs – 1 cycle: denaturation at 94 °C − 3 min; 35 cycles: denaturation (94 °C) − 1 min, annealing − 1 min (65 °C for TGF-β1; 58 °C for PIT-1; 53 °C for IGF-I), elongation (72 °C) − 1 min; 1 cycle: final elongation (72 °C) − 10 min. The volume of the final mixture was 20 μl, the concentration of primers – 0.2 μM in each case.

The amplified fragments were treated with restriction endonucleases *MboII* (restriction site GAAGA (8/7)↓) and *PstI* (restriction site CTGCA↓G) according to the standard methods of the manufacturer (ThermoScientific). In case of TGF-β1 gene *MboII* was used, and for IGF-I – *PstI*. The restriction analysis was not conducted to study polymorphism by locus PIT-1. The products of amplification/restriction were separated in 1.5 % agarose gel at 150 V for 40 min. The visualization was conducted using ethidium bromide in the ultraviolet spectrum. The size of the amplified/restrictional fragments was determined using the markers of molecular mass M-50 and M-100 (Isogen, RF).

The genotyping by each locus was conducted using the analysis of the electrophoregrams obtained.

Gene TGF-β1: the size of the amplified fragment – 240 bp. Genotype B/B was presented on the electrophoregram in the form of DNA fragments of 173 and 67 bp; F/F – 240 bp; B/F – 240, 173 and 67 bp respectively.

Gene PIT-1: fragments of 387 bp are present on the electrophoregram, which testifies to genotype I/I, and 330 bp – D/D. Heterozygote chickens I/D are present in the combination of both variants – 387 and 330 bp respectively.

Gene IGF-I: the size of the amplified fragment – 621 bp. Genotype C_1/C_1 on the electrophoregram corresponds to the fragment of 621 bp; C_2/C_2 – 257 and 364 bp; C_1/C_2 – three fragments of 621, 257 and 364 bp respectively.

The frequency of alleles of polymorphic loci was determined by the formulas of maximum likelihood. The data obtained was used for the calculation of actual and theoretical distribution of genotypes, the correspondence to genetic equilibrium of the population by Hardy-Weinberg, using criterion χ^2, actual (H_o) and expected (H_e) heterozygosity, efficient number of alleles (n_e), Wright's fixation index (F_{is}) using the Popgen32 program. The productivity traits of chickens were also taken into consideration: the number of eggs in 12 weeks of productivity (En_{12}); the number of eggs in 40 weeks of productivity (En_{40}); the weight of eggs on the 30th week of life (Ew_{30}); the weight of eggs on the 52nd week of life (Ew_{52}); live weight; the weight of eviscerated carcass, pectoral muscles, muscles of legs, thighs, gizzard stomach, liver, heart, and visceral fat.

RESULTS AND DISCUSSION

The use of the molecular and genetic analysis allowed revealing the variability of each gene, studied in the current work – TGF-β1, PIT-1 and IGF-I.

The polymorphism of TGF-β1 gene is related to the transversion of cytosine in position 632 – missence mutation, leading to the substitution of glutamin for asparagine in the encoded protein, which results in the occurrence of the restriction site for *MboII* in allele B.

The allele variants of TGF-β1 gene, occurring due to *MboII*-polymorphism of the exon region, are presented on the electrophoregram of restriction products (Fig. 1).

The data obtained present evidence to the fact that TGF-β1 gene is polymorphic in the studied population of chickens. There are individuals of all the possible genotypes (B/B, B/F, F/F).

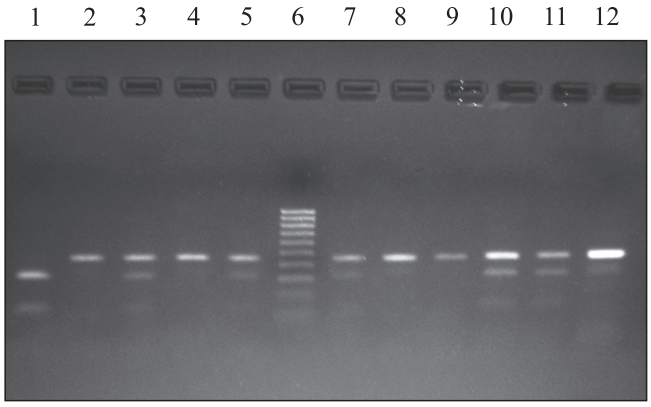

Fig. 1. The electrophoregram of restriction products of the exon region of TGF-β1 gene: *1* – genotype B/B; *2*, *4*, *8*, *9*, *12* – genotype F/F; *3*, *5*, *7*, *10*, *11* – genotype B/F; *6* — marker of molecular mass M-50

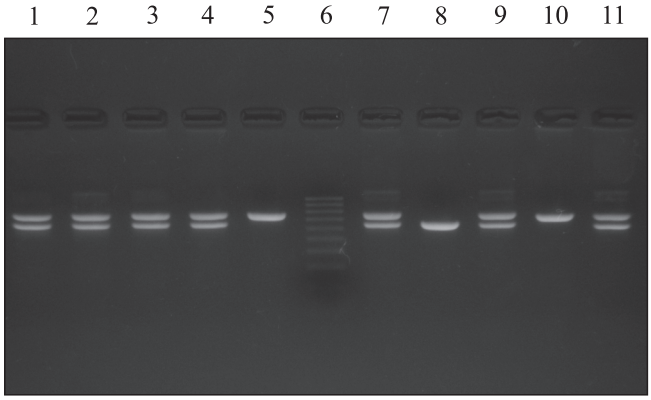

Fig. 2. The electrophoregram of the amplification products of the second intron of PIT-1 gene: *1–4*, *7*, *9*, *11* – I/D; *5*, *10* – I/I; *8* – D/D; *6* – marker of molecular mass M-50

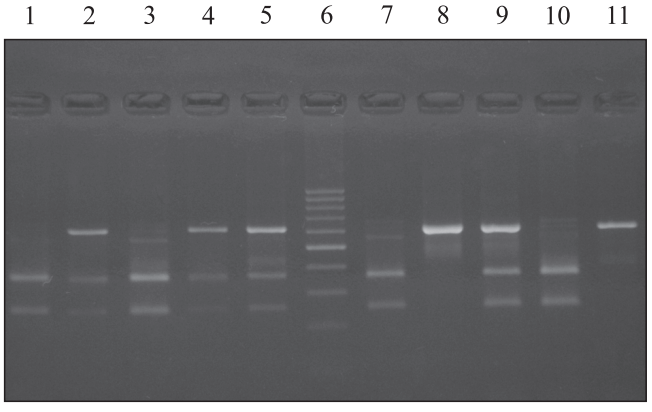

Fig. 3. The electrophoregram of restriction products of amplified fragment of IGF-I gene: *1*, *3*, *7*, *10* – C_2/C_2; *2*, *4*, *5*, *9* – C_1/C_2; 8, 11 – C_1/C_1; *6* – marker of molecular mass M-100

The polymorphism of the gene is related to the presence of the insert of 57 bp in the second intron, which leads to the formation of two allele variants –

I and D. Figure 2 presents the electrophoregram of the amplification products of the second intron of PIT-1 gene.

By the presence of the insert in the second intron PIT-1 gene is also polymorphic in the studied population of chickens. There are chickens of all three possible genotypes: I/I, I/D and D/D.

The allele variants of IGF-I gene occur due to the transition of cytosine into thymine which results in the loss of restriction site for *PstI* in case of alle-

Table 1. The genetic structure of the population of Poltava clay chicken breed by loci TGF-β1, PIT-1, and IGF-I

Locus	Genotype frequency			Allele frequency		χ^2
TGF-β1	B/B 0.1	B/F 0.43	F/F 0.47	B 0.31	F 0.69	0.03
PIT-1	I/I 0.38	I/D 0.5	D/D 0.12	I 0.63	D 0.37	0.62
IGF-I	C1/C1 0.17	C1/C2 0.42	C2/C2 0.41	C1 0.38	C2 0.62	1.17

Table 2. The association of different genotypes by loci TGF-β1, PIT-1 and IGF-I and the indices of egg productivity of Poltava clay chicken breed

Gene	Genotype	Index			
		En_{12} (it.)	En_{40} (it.)	Ew_{30} (g)	Ew_{52} (g)
TGF-β1	B/B	67.0 ± 2.91[a]	173.7 ± 8.13[a]	49.1 ± 1.32[a]	56.8 ± 2.68[a]
	B/F	66.5 ± 1.84[a]	190.2 ± 4.32[a]	51.9 ± 0.69[a]	58.7 ± 0.75[a]
	F/F	67.8 ± 1.18[a]	193.2 ± 2.38[b]	51.9 ± 0.56[a]	58.6 ± 0.68[a]
PIT-1	I/I	66.6 ± 1.62[a]	184.2 ± 5.06[a]	51.1 ± 0.63[a]	57.2 ± 0.72[a]
	I/D	68.4 ± 1.13[a]	191.7 ± 3.25[a]	51.8 ± 0.57[a]	58.9 ± 0.69[a]
	D/D	69.8 ± 2.44[a]	195.0 ± 7.03[a]	51.4 ± 1.61[a]	60.6 ± 2.15[a]
IGF-I	C1/C1	65.9 ± 2.55[a]	186.5 ± 6.32[a]	53.6 ± 1.28[a]	58.1 ± 1.59[a]
	C1/C2	68.1 ± 1.31[a]	190.2 ± 5.05[a]	50.9 ± 0.64[a]	58.3 ± 0.79[a]
	C2/C2	68.4 ± 1.49[a]	189.6 ± 4.39[a]	51.1 ± 0.64[a]	58.5 ± 0.77[a]

Note. [a, b]Differences are reliable ($p < 0.05$) within the locus.

Table 3. The association of different genotypes by loci TGF-β1, PIT-1 and IGF-I and the indices of meat productivity of Poltava clay chicken breed

Gene	Genotype	Live weight, kg	Eviscerated carcass, kg	Pectoral muscles, g	Thigh muscles, g	Leg muscles, g	Gizzard stomach, g	Visceral fat, g
TGF-β1	B/B	2.21 ± 0.070[a]	1.37 ± 0.065[a]	112.7 ± 5.17[a]	77.9 ± 3.46[a]	59.1 ± 1.89[a]	32.2 ± 1.08[a]	54.1 ± 10.97[a]
	B/F	2.46 ± 0.051[ab]	1.44 ± 0.041[b]	116.2 ± 2.99[a]	82.3 ± 2.46[ab]	64.00 ± 1.63[ab]	35.1 ± 1.07[b]	43.3 ± 4.27[a]
	F/F	2.46 ± 0.046[b]	1.56 ± 0.039[c]	120.3 ± 2.77[a]	88.0 ± 2.03[b]	66.5 ± 1.44[b]	35.2 ± 0.91[b]	52.2 ± 3.32[a]
PIT-1	I/I	2.32 ± 0.049[a]	1.44 ± 0.037[a]	116.6 ± 3.19[a]	83.6 ± 2.52[a]	64.4 ± 1.60[a]	34.3 ± 0.98[a]	45.7 ± 3.95[a]
	I/D	2.40 ± 0.044[a]	1.50 ± 0.037[a]	117.9 ± 2.44[a]	83.3 ± 1.97[a]	63.5 ± 1.33[a]	35.2 ± 0.87[a]	48.0 ± 3.52[a]
	D/D	2.53 ± 0.165[a]	1.61 ± 0.140[a]	120.2 ± 9.20[a]	96.8 ± 4.43[b]	73.2 ± 3.52[b]	36.3 ± 3.34[a]	60.3 ± 17.48[a]
IGF-I	C1/C1	2.44 ± 0.095[a]	1.55 ± 0.067[a]	115.7 ± 4.87[a]	88.9 ± 4.74[a]	65.5 ± 2.69[a]	34.9 ± 1.82[a]	59.8 ± 6.49[a]
	C1/C2	2.36 ± 0.048[a]	1.48 ± 0.040[a]	119.6 ± 3.05[a]	83.7 ± 2.19[a]	64.6 ± 1.64[a]	34.4 ± 0.96[a]	44.7 ± 3.72[b]
	C2/C2	2.36 ± 0.042[a]	1.46 ± 0.042[a]	116.2 ± 2.77[a]	82.8 ± 2.13[a]	63.9 ± 1.44[a]	35.5 ± 0.97[a]	46.5 ± 4.48b

Note. [a, b, c]Differences are reliable ($p < 0.05$) within the locus.

le C_1. The indication of alleles (C_1 and C_2) is related to the number of cytosine residues in the restriction site for *PstI*. The electrophoregram of restriction products (*PstI*) of 5'UTR fragment of IGF-I gene is presented in Fig. 3.

The studies demonstrated that similar to the abovementioned cases IGF-I locus is polymorphic in the experimental population of chickens. There are chickens of all the possible genotypes: C_1/C_1, C_1/C_2 and C_2/C_2. The genetic structure of the experimental population of chickens for all three studied loci is presented in Table 1.

The values of the actual heterozygosity by different loci in the experimental population of chickens were in the range of 0.42–0.5 while the values of expected heterozygosity were from 0.428 to 0.471. The highest level of H_e was observed by locus IGF-I (0.471), the lowest – by TGF-β1 (0.428). Some deficiency of heterozygous chickens was defined by locus IGF-I (F_{is} = 0.108). In its turn some excess of heterozygotes was observed by locus PIT-1 (F_{is} = −0.07). The value of the efficient number of alleles fluctuated in the range of 1.75–1.89, and the highest level of polymorphism in the studied population was noted for locus IGF-I.

The analysis of actual and theoretical distribution of chickens of different genotypes (by all the studied loci) in the experimental population of chickens did not reveal any disorder of genetic equilibrium which testifies to the absence of any selection pressure.

The presence of chickens of all the genotypes, possible for each locus, in the experimental population allowed investigating the connection of different genotypes to the productivity traits of chickens.

As for locus TGF-β1, it was demonstrated that the egg productivity of chickens with genotype F/F in 40 weeks exceeded that of chickens with genotype B/B and amounted to 193.2 ± 2.38 and 173.7 ± 8.13 ($p < 0.05$) (Table 2).

It was also noted that by locus PIT-1 the egg productivity of chickens with genotype D/D for the same time period was 195.0 ± 7.03, which was more than the index for chickens with genotype I/I – 184.2 ± ± 5.06. However, in this case the difference is not reliable which is considerably related to a small number of chickens with genotype D/D. As for the rest of the loci, no significant differences in the indices of egg productivity were revealed between the chickens of different genotypes.

There are no remarkable differences for each locus by the egg weight indices on the 30th and 52nd weeks of life.

Reliable differences were revealed in the meat productivity of chickens by loci TGF-β1 and PIT-1 (Table 3).

It was demonstrated that by locus TGF-β1 chickens with genotype F/F are characterized by reliably higher values of indices of live weight, mass of eviscerated carcass, muscles of thigh, legs and gizzard stomach compared against chickens of genotype B/B (Table 3).

In their turn some reliable differences between the chickens of genotypes D/D and I/I by the indices of the weight of muscles of thigh and legs were registered for locus PIT-1. There is also a tendency of prevalence of genotype D/D chickens by the indices of live weight and the mass of eviscerated carcass. At the same time there were no reliable differences for locus IGF-I by the studied indices, except for the index of visceral fat mass (chickens of genotype C_1/C_1 are reliably exceeding chickens of genotype C_1/C_2).

There were no reliable differences between the values of the weight of heart and liver for any locus.

CONCLUSIONS

The genetic structure of Poltava clay chicken breed was studied by loci of transforming growth factor β1, pituitary transcriptional factor 1 and insulin-like growth factor I. It was determined that all the investigated loci in the experimental population of chickens were polymorphic. The association between genotypes by the loci TGF-β1 and PIT-1 and the indices of egg and meat production of chickens was demonstrated. The data obtained are recommended for the application in the targeted selection of Poltava clay chicken breed with the purpose of obtaining the offspring of desired genotypes which will promote maximum efficiency in realizing productive potential of poultry in addition to classic selection methods.

Поліморфізм генів трансформуючого ростового фактора β1, гіпофізарного фактора транскрипції 1 та інсуліноподібного ростового фактора I у популяції курей породи полтавська глиняста: зв'язок з продуктивними ознаками

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Мета. Дослідження поліморфізму генів трансформуючого ростового фактора β1 (TGF-β1), гіпофізарного фактора транскрипції 1 (PIT-1) та інсуліноподібного ростового фактора I (IGF-I) у популяції курей яєчно-м'ясного напрямку продуктивності породи полтавська глиняста та аналіз зв'язку різних генотипів за кожним

локусом з продуктивними ознаками. **Методи.** Особин генотипували з використанням полімеразної ланцюгової реакції та аналізу поліморфізму довжини рестрикційних фрагментів (ПЛР-ПДРФ). **Результати.** Показано, що в популяції курей породи полтавська глиняста локуси TGF-β1, PIT-1 та IGF-I є поліморфними. Встановлено зв'язок генотипів за локусами TGF-β1 і PIT-1 з показниками яєчної та м'ясної продуктивності. **Висновки**. Отримані дані щодо генетичної структури популяції курей породи полтавська глиняста за локусами TGF-β1 і PIT-1 рекомендовано використовувати при здійсненні спрямованої селекції курей для отримання нащадків з бажаними генотипами, що дозволить (як доповнення до класичних селекційних методів) максимально ефективно розкрити продуктивний потенціал птиці.

Ключові слова: порода полтавська глиняста, поліморфізм, трансформуючий ростовий фактор β1, гіпофізарний фактор транскрипції 1, інсуліноподібний ростовий фактор I, продуктивні ознаки.

Полиморфизм генов трансформирующего ростового фактора β1, гипофизарного фактора транскрипции 1 и инсулиноподобного ростового фактора I в популяции кур породы полтавская глинистая: связь с продуктивными признаками

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Цель. Исследование полиморфизма генов трансформирующего ростового фактора β1 (TGF-β1), гипофизарного фактора транскрипции 1 (PIT-1) и инсулиноподобного ростового фактора I (IGF-I) в популяции кур яично-мясного направления продуктивности породы полтавская глинистая и анализ связи различных генотипов по каждому из локусов с продуктивными признаками. **Методы.** Особей генотипировали с использованием полимеразной цепной реакции и анализа полиморфизма длины рестрикционных фрагментов (ПЦР-ПДРФ). **Результаты.** Показано, что в популяции кур породы полтавская глинистая локусы TGF-β1, PIT-1 и IGF-I являются полиморфными. Установлена связь генотипов по локусам TGF-β1 и PIT-1 с показателями яичной и мясной продуктивности кур. **Выводы.** Полученные данные о генетической структуре популяции кур породы полтавская глинистая по локусам TGF-β1 и PIT-1 рекомендуется использовать при проведении направленной селекции кур для получения потомства с желательными генотипами, что позволит (в дополнение к классическим селекционным методам) максимально эффективно раскрыть продуктивный потенциал птицы.

Ключевые слова: порода полтавская глинистая, полиморфизм, трансформирующий ростовой фактор β1, гипофизарный фактор транскрипции 1, инсулиноподобный ростовой фактор I, продуктивные признаки.

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