Growth Evaluation of the Slovak National Chicken Breed Oravka Depending on the Melanocortin Receptor 4 Gene

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Abstract. *We designed and tested the polymorphic gene MC4R in relation to live weight and weight gain of Slovak native chicken breed Oravka kept at the farm of NAFC-RIAP Nitra, which was selected for future breeding by phenotype. We monitored the weight of chickens at the age of 5, 12 and 20 weeks and calculated the weight gain for each period. The design of detection oligonucleotide primers for MC4R gene detection in DNA samples was based on the sequence available in GenBank, NCBI. Statistical analysis was done using the SAS 9.2 Stat software. We identified the GG genotype in only one hen. The number of GT heterozygotes was significantly higher in both hens (110) and roosters (16) after a long-time directive selection. The TT genotype was found in 9 hens and 5 roosters. The GG genotype was detected with a frequency of 0.71%, the GT genotype with a frequency of 89.36%, and the TT genotype with a frequency of 9.93%. The frequency of allele G was 0.454 ± 0.030; and that of allele T was 0.546 ± 0.030. At the age 12 weeks, homozygous hens were heavier (1111.33 ± 44.96 g) than heterozygous hens (1108.15 ± 13.17 g). Similarly, rooster homozygotes were on average heavier (1581.47 ± 59.11 g) than heterozygotes (1389.69 ± 34.40 g). WG 1 was significantly higher in homozygote animals TT ($P < 0.01$). The same pattern was also observed at the age of 20 weeks. Homozygous TT animals achieved a higher average weight, i.e., the weight of hens was 1947.25 ± 74.35 g, the weight of roosters was 2719.91 ± 87.75 g; meanwhile, for heterozygous animals, the weight of hens was 1888.78 ± 21.78 g, and the weight of roosters was 2525.99 ± 56.89 g. We concluded that in the selected Oravka "mutant" allele T is preferred.*

Introduction

Current trends in food production are increasingly shifting from a quantitative to a qualitative position, i.e., towards the production of quality and safe food. This trend is mainly related to new knowledge about the rational nutrition of the population and, thus, also to the increasing interest of the population in these foods. The basic scientific approach to the production of food of animal origin is the precise characterisation and evaluation of the genetic potential of farm animals. In addition to monitoring reproduction, performance indicators and product quality, modern molecular genetics and immunological methods are used for comprehensive evaluation. These methods allow the identification of prognostically relevant biomarkers and gene expression profiles that will help to better understand the complex molecular mechanisms of cell physiology and pathology with their subsequent application in selection for higher production efficiency (higher growth rate, carcass yield, reproduction) and product quality.

A strategically important aspect in the field of animal production research is the potential to use original local breeds and farm animal lines. In Slovakia, these are currently maintained mainly by breeders organised by the Slovak Breeders' Association and specialised breeders' clubs acting under the umbrella of this organisation. Long-term one-way selection focused on the exterior characteristics of these breeds gradually leads to a significant reduction in their reproductive (low number of litters, poor viability) but also production characteristics, which belong to the typical characteristics of their breeding standards. The continuation of such an organisation of their breeding threatens the gradual extinction of already endangered populations of these breeds. The knowledge of genetic polymorphism influencing the production traits significantly contributes to streamlining the sustainability process of national breeds and lines. The Slovak native poultry breed Oravka belongs to the animal genetic resources rearing at the National Agricultural and Food Centre in Nitra (Slovak Republic). Detailed knowledge of the molecular polymorphism of the melanocortin receptor 4 (*MC4R)* gene, which significantly affects the production (growth and slaughter) properties of poultry, will make it possible to refine and objectify the breeding standard and streamline the sustainability process of the breed.

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Body weight is one of the most important economic traits in the poultry industry. It is well known that the birds' feed intake regulation and the central melanocortin system are influenced by *MC4R* gene. In addition, the regulation of melanocytic pigmentation and energy homeostasis is achieved by melanocortins that are peptide hormones derived from proopiomelanocortin (Boswell and Takeuchi, 2005). The current bibliography in animal models underlines the essential role of *MC3R* and *MC4R* in the regulation of feeding and energy homeostasis (Schwartz et al., 2000). A significant correlation between the *MC4R* genotype and body weight has been identified (Li and Li, 2006; Qiu et al., 2006; Wang et al., 2009). In humans and mice, *MC4R* has been recognised as a major regulator of food intake, body weight, and glucose homeostasis (Wardlaw, 2001; Cone, 2006). A significant association between MC4R SNPs and growth traits has been found in several farm animals, including chickens (Kim et al., 2000; Qiu et al., 2006; Wang et al., 2009; Zhang et al., 2009). In mammals, *MC4R* is associated with appetite, body weight, and energy metabolism (Andersson, 2003), and it can also mediate the function of leptin (Hoggard et al., 2004). Changes in protein structure caused by different *MC4R* genotypes are expected to affect the activity or function of proteins related to body weight in chickens (Li and Li, 2006).

The melanocortin receptor plays an important role in the central melanocortin system (CMS) and muscle cells. It is a member of the so-called G-protein receptor family, which is expressed in the hypothalamus and is involved in the control of energy homeostasis and food intake with a major impact on body weight and fat storage. The chicken *MC4R* gene is located on chromosome 2. In humans, the occurrence of heterozygous mutations in *MC4R* is associated with dominant inherited obesity and is the most common monogenic cause of human obesity. *MC4R* has been found to be involved in food intake, metabolic regulation, and body weight (Li and Li, 2006). Significant association relationships between mutations in this gene, feed intake intensity, and live weight gain have been demonstrated in many animal species (Zhang et al., 2009). Mutations in the *MC4R* gene are also associated with carcass quality in cattle (Zhang et al., 2009) and broilers (Wang et al., 2009). El-Sabrout and Aggag (2019) described a direct relationship of polymorphism in *MC4R* with live weight gain intensity and some behavioural functions of rabbits. For these reasons, it has been included as a significant candidate gene for marker-assisted selection (MAS) related to the production characteristics of rabbits and poultry, such as live weight gain, feed conversion and carcass yield.

Holsinger and Weir (2009) described a big number of single nucleotide polymorphisms in the genomes of several animal species, which allowed the study of the entire genome and the effective setting of selection by evaluating different allele frequencies between populations. Genes associated with production traits were identified using single nucleotide polymorphisms and included in the group of so-called candidate genes (Wu et al., 2015; Zhang et al., 2013).

The current economic pressure in poultry production is causing interest in specialised poultry lines focused on laying or meat performance in developed countries. Continued genetic improvement of livestock depends on the genetic variability that exists within individual breeds (Sruoga et al., 2007). Quantitative assessment of genetic variability within and between populations is an important tool in developing plans for the conservation of genetic resources. Understanding the associations between genotype and phenotype is very important in terms of faster progress in animal husbandry. Results derived from molecular genetic studies play an important role in breeding value prediction systems and in the generation of specialised lines and animal populations. The body composition, fat storage and meat quality are important indicators of poultry. The *MC4R* gene in chickens is associated with carcass yield and meat quality.

In general, two alleles-nucleotides are maintained in position 923 of the MC4R-AY545056 gene, the original G and mutant T in the chicken population. Mutations in the melanocortin-4 receptor *MC4R* gene are associated with appetite, obesity, and growth in pigs, mice, and humans. However, little is known about the function of the chicken *MC4R* gene. Qiu et al. (2006) tested F2-generation broiler chickens for *MC4R* gene polymorphisms using single-stranded conformational polymorphism PCR (PCR-SSCP) and DNA sequencing, founding four sites of single nucleotide polymorphisms (SNPs). The results provide evidence that the chicken MC4R gene can be selected as a major candidate gene for carcass traits such as body weight and growth. Molee et al. (2018) examined the association between different genotypes of the *MC4R* gene in native chickens in Thailand and found no significant relationship between this gene and live weight. Davies et al. (2002) reported that sense mutation can change the gene expression, which in turn leads to a different protein with different characteristics as a result of amino acid change. This protein may lose its function or become activated or exhibit a new function. It is possible that this variation causes a significant change of the *MC4R* function. Amino acid change may also affect the biosynthesis of other nutrients. It can stimulate the feed intake, metabolism and growth of eggs, which in turn affects the egg weight. This finding is in agreement with results obtained by El-Sabrout and Aggag (2017), who found that *MC4R* plays an area responsible for controlling feed intake behaviour, which in turn affects the body weight.

The aim of this study was to design a PCR-based

technique to detect and test specific single nucleotide polymorphisms (SNPs) in gene *MC4R* of Slovak native chicken Oravka. An innovative dimension of this study is the application of outputs and results for identifying critical populations and a breeding selection programme for the preservation of the local well-adapted hen breed. The further aim was to investigate the influence of some factors (genotype, year, sex) on the variation of live body weight and weight gain.

Material and methods

Animals

The animal genetic resources of yellow-brownish Oravka breed (hens and roosters) are kept at the farm of the National Agriculture and Food Centre (NPPC) – Research Institute for Animal Production Nitra (Slovak Republic), considered as the *ex situ* flock, and included in the experiment which was performed during three consecutive years (2018 to 2020). From all hatched chickens, only animals that had the breed standard were selected for breeding. We monitored the weight of chickens at the age of 5, 12 and 20 weeks (LW 5, LW 12, LW 20) using the BAT 1 manual poultry scale (produced by VEIT Electronics, Czech Republic). From the obtained values, we calculated the weight gain for each period (weight gain between week 5 and week 12 – WG1; weight gain between week 12 and week 20 – WG2). Health was monitored daily. At the age of 12 weeks, when the animals are selected for breeding, the wing feathers of chickens were taken for genetic analysis. Birds were kept in closed heating nurseries on deep litter until 5 weeks of age; afterwards, they were housed in a heatless hen-house with a covered yard and free range. Birds were fed (ad libitum) a complete feed (ME 2600 kcal/kg, 16% crud protein) for light laying hens (according to age categories); water was available during the whole experiment.

Molecular-genetics analysis

Oligonucleotides for ARMS-PCR for *MC4R* gene detection in DNA samples were designed based on used tools like Primer-BLAST on GenBank, NCBI (Gene bank no. NC_052533, last access 15 March 2024). For ARMS primers, the 3' end of allele-specific primers was manually adjusted to align with the SNP, ensuring the specificity for either allele. Proposed diagnostic MC4R-GAL -ARMS-PCR oligonucleotide sequences were MC4R-GAL-ARMS-OMHZ-G-FOR with the sequence 5´-AATGGATCAGAAACTATTGTCATCACGCTG–3´, primer MC4R-GAL-ARMS-OMHZ-T-FOR with the sequence 5´-AATGGATCAGAAACTATTGTCATCACGCTT – 3´, and primer MC4R-GAL-ARMS-OMHZ-G+T REV with the sequence 5´-CTGGAGGGCATAAAAGATAG – 3´.

Processing and preparation of biological samples

The biological samples were taken in a non-invasive manner (pulp from the growing feathers) from poultry of the yellow brownish Oravka breed. DNA isolation from biological samples was performed using commercially available automatic DNA isolation kit MagNA Pure LC DNA Isolation kit II (Tissue) and Tissue kit for manual isolation (Macherey-Nagel, NucleoSpin Tissue) following the manufacturers' instructions.

Subsequently, specific selected sections of the *MC4R* gene were amplified using specific oligonucleotides designed by our site.

Analysis of DNA polymorphisms of selected genes

Molecular genetic analyses were performed at the laboratory of the Department of Nutrition and Small Farm Animals, NPPC, using a Bio-Rad T100 gradient PCR thermocycler. PCR conditions were 95°C for 2 minutes, 95°C for 30 s, 60.7°C for 30 s, 72°C for 30 s, 35 cycles, with the last extension step at 72°C for 10 minutes. The PCR reaction volume (25µL) contained a mastermix: 10 mM Tris-HCl (pH 8.6 at 25°C, 50 mM KCl, 1.5 mM MgCl2, 25 units / mL Taq DNA polymerase, 0.2 mM dNTPs, 5% glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween-20 – New England Biolabs), primers (MC4R-GAL-ARMS G-FOR, MC4R-GAL-ARMS T-FOR, MC4R-GAL-ARMS G+T REV) at a concentration of 10 pmol/L. In the case of poultry, we detected a fragment with a total length of 180 bp. The polymorphism of the *MC4R* gene was determined using ARMS-PCR (PCR analysis using allele-specific oligonucleotides), followed by electrophoretic separation and visualisation using a MiniBis Pro photodocumentation device (Bio-Imaging Systems) with in situ analysis using GelQuant Express software, so that the original methodology shortened the whole analysis and made the whole process more economical. PCR products were then electrophoretically separated on a 3% agarose gel containing ethidium bromide at 80 V, 60 mA in 10 mM lithium borate buffer for 60 minutes and then visualised under UV light (Fig. 1) and photographed using MiniBis Pro documentation system (Bio-Imaging Systems).

ARMS-PCR (Amplification Refractory Mutation System-PCR) is used for genotyping SNP with the help of refractory primers. Designing primers for the mutant (with SNP) and normal (without SNP) alleles allows selective amplification, which can be easily analyzed after electrophoresis. Modification of a single base occurs at the 3' end of the primer such that one primer matches the normal allele (G) and the other matches the mutant allele (T). Both primer variants are combined in a single PCR reaction mixture so that the PCR is performed simultaneously. By running two PCR reactions (one with a mutant-specific primer and one with a wild-type-specific primer), the presence or absence of specific amplification products

can reveal whether the sample contains the wild-type, mutant, or both alleles (heterozygous condition). The modification of primers is the most crucial highlight in the mechanism of ARMS-PCR. Selective amplification happens due to the mismatch induced for primer variants. Introducing a mismatch at the 3' end of the primer alters the annealing temperature for the allele variant. Since Taq DNA polymerase is unable to perform exonuclease activity, the mismatch cannot be repaired. The general procedure of the ARMS-PCR involves four major steps: primer design, amplification, electrophoresis, and results.

For primer design, our DNA sequence, for example, features a G in the normal allele and T in place of G in the mutant allele. Designing a forward primer for the normal allele should contain G while the mutant allele contains T in place of G at the 3' end. The success of ARMS-PCR relies on the presence of an added mismatch base near to the SNP at the 3' end. The reverse primer generally remains the same. Moreover, the primer must still fulfil all the criteria for the ideal primer like in a normal PCR.

Genotype GG: at the G allele position, there is one fragment of 180 bp; the T allele position is without a fragment.

Genotype TT: at the T allele position, there is one fragment of 180 bp; the G allele position is without a fragment.

Genotype GT: at both positions of the G and T alleles, there are fragments of 180 bp.

The Sanger sequencing method (Merck KGaA, Darmstadt, Germany) was used to confirm the SNP in the observed fragment during the DNA sequencing. DNA sequence is determined by synthesising a complementary strand of DNA, a template DNA strand, and a primer. During the synthesis of the complementary strand, the DNA polymerase incorporates fluorescently labelled nucleotides at positions where they match the template DNA strand. Formed DNA strands are size separated through gel electrophoresis, with the sequence's determination being reliant on the colour of the fluorescent label at each position. Figures 2, 3 and 4 show the complementary sequences of the DNA strand to the region detected by ARMS-PCR with the target complementary base indicated.

Statistical analysis

Based on molecular genetic analysis and mathematical-statistical calculations from the observed observations of live weight and live weight gain, association studies were performed in relation to the monitored growth parameters of poultry. Statistical analysis was done using the SAS 9.2 statistical software (2009). The GLM model was applied to study the influence of genotypes, year, sex and sex*genotype on live weight and weight gain of Oravka chicken.

The following model was applied:

$$Y_{ijk} = \mu + G_i + Y_j + Sk + e_{ijk},$$

where Y_{ijk} – individual live body weight /weight gain parameters; μ – intercept; G_i – fixed effect of genotype (GT, TT); $\Sigma_i G = 0$; Y_j – fixed effect of year (18, 19, 20); $\Sigma_j Y = 0$; Sk – fixed effect of sex (F, M); $\Sigma k\ S = 0$; e_{ijk} – random error.

Fixed effects included in the model were estimated using the Least Squares (LSM) method. Statistical significances of fixed effects were tested by the Fischer F test; statistical significances of individual differences between estimated levels of fixed effects were tested by the Scheffe multiple range tests. Differences were considered significant when $P < 0.05$.

Results and discussion

In the presented study, we focused on the screening of the population of the national breed of hens – yellow brownish Oravka bred at NPPC Nitra (Slovakia). The candidate melanocortin receptor 4 gene was included for screening as a potential marker for assisted selection (MAS) related to poultry production traits such as live weight and average weight gain.

The results of genotyping of reference samples (Fig. 1) suggested that, in the selected population of standard Oravka, the *GG* genotype demonstrated a frequency of 0.71%, the *GT* genotype showed a frequency of 89.36%, and the *TT* genotype showed a frequency of 9.93%. The frequency of the original *G* allele was 0.454 ± 0.030 and the frequency of the mutant allele *T* was 0.546 ± 0.030. These results were confirmed by the Sanger sequencing method (Fig. 2, Fig. 3A, Fig. 3B, and Fig. 4) indicating the presence of selected SNP in the observed fragment during the DNA sequencing.

Similarly to this study, Molee et al. (2018) detected the higher frequency of the T allele: T = 0.668 compared with the G allele (0.332) in indigenous rapid growth rate chicken in Thailand. They investigated 510 chickens and found three genotypes with the following frequencies: *GG* = 0.24; *GT* = 0.18; *TT* = 0.58.

Table 1 shows the general linear model for live weight and weight gain of 140 animals. With respect to the coefficient of determination, the model explained the variation of evaluated variables between 39% (weight gain 2) and 62% (live weight 20). The adjustment for fixed factors (genotype, year, sex and interaction between genotype and sex) decreased the variation by 22% (live weight 5), by 37% (live weights 12 and 20), by 34% (weight gain 1) and by 80% (weight gain 2).

Least-square means and standard errors, estimated within the effects of genotype, year and sex for live weight at 5, 12 and 20 weeks of age and for weight gain between 5 and 12 weeks of age and 12 and 20 weeks of age are given in Table 2. At the beginning of the experiment, at the age of 5 weeks, average live weight was higher in heterozygote animals GT

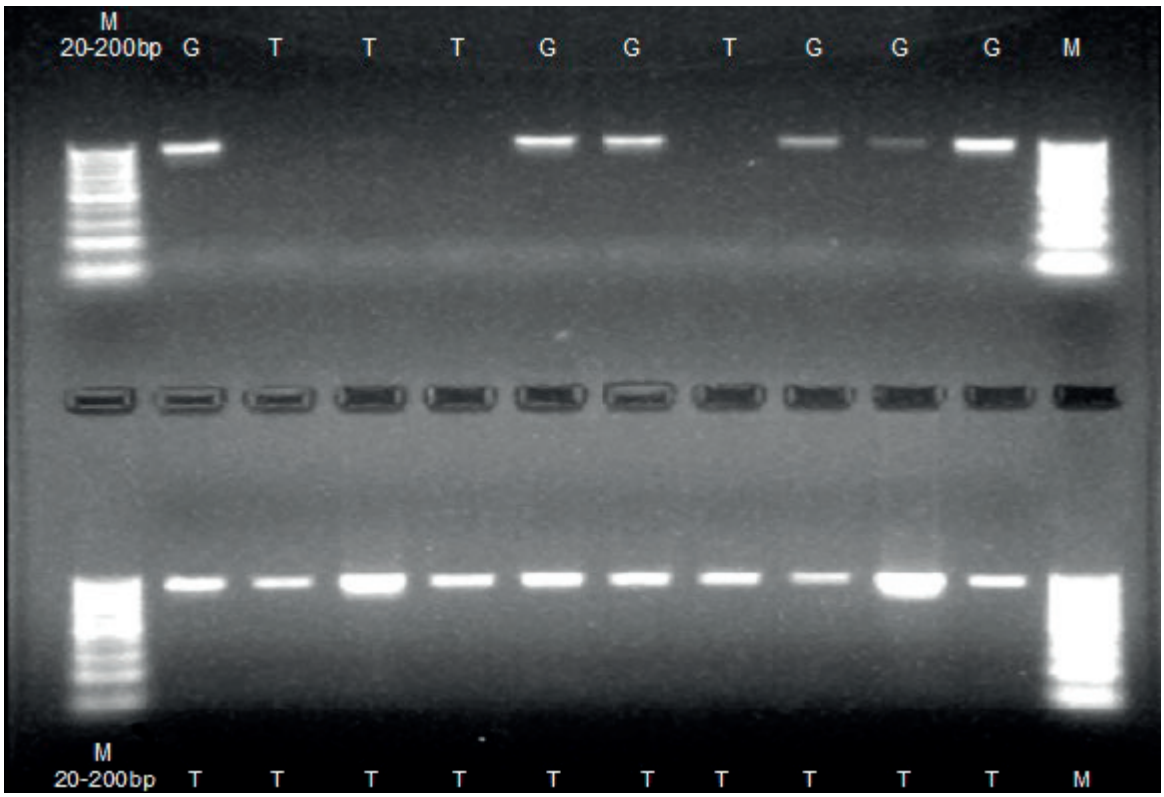

Fig. 1. Electrophoretogram of polymorphism with analysis of *MC4R* genotypes GT and TT of reference samples of Oravka breed.

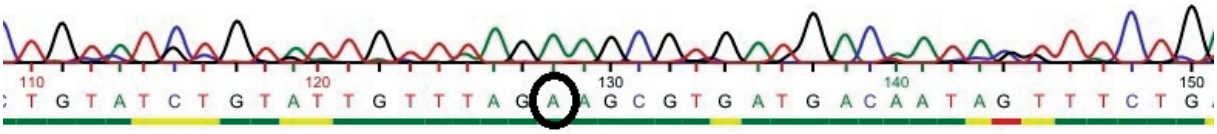

Fig. 2. Complementary base "A" (in the circle) in a DNA chromatogram of PCR products based on the Sanger sequencing method – *MC4R* genotype T.

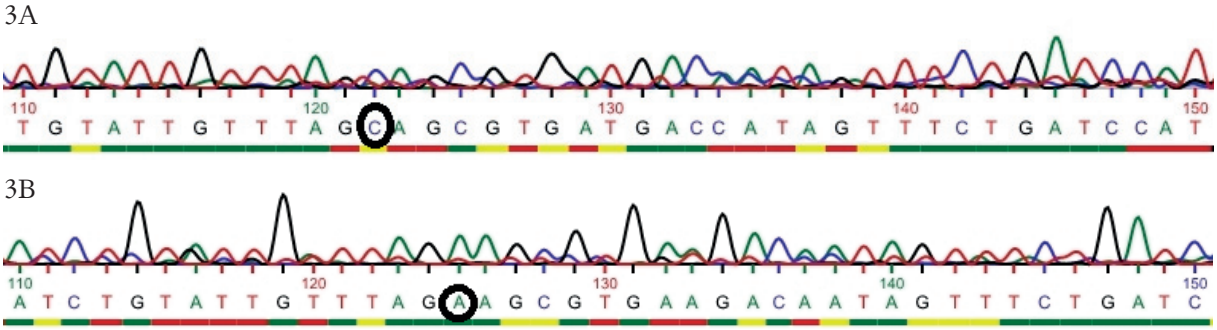

Fig. 3A–3B. Complementary bases "C" and "A" (in the circle) in a DNA chromatogram of PCR products based on the Sanger sequencing method – *MC4R* genotype GT.

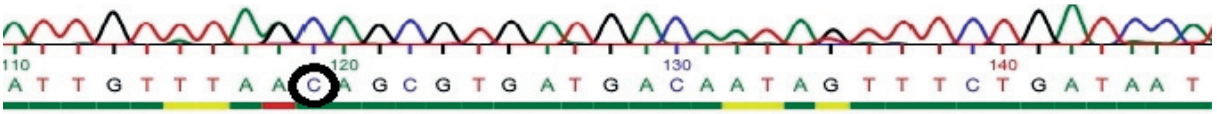

Fig. 4. Complementary base "C" (in the circle) in a DNA chromatogram of PCR products based on the Sanger sequencing method – *MC4R* genotype GG.

Table 1. Selected characteristics of the general linear model for live weight (g) and weight gain (g), N = 140

	Live weight 5	Live weight 12	Live weight 20	Weight gain 1	Weight gain 2
R2	0.419	0.611	0.617	0.583	0.385
RMSE	62.226	131.970	218.221	115.606	162.570
Mean	470.286	1122.929	1952.286	652.643	829.357
STD	80.1	207.9	346.3	175.8	203.5

*R*2 – coefficient of determination; RMSE – Root means squares error; STD – standard deviation.

Table 2. Least-squares means and standard errors for live weight and weight gain

Effect		LW 5 (g)	LW 12 (g)	LW 20 (g)	WG 1(g)	WG 2(g)
	N	μ ± sμ	μ ± sμ	μ ± sμ	μ ± sμ	μ ± sμ
Genotype						
GT	126	501.19 ± 8.88	1248.92 ± 18.83a	2207.38 ± 31.14	747.73 ± 16.50a	958.46 ± 23.20
TT	14	494.50 ± 17.56	1346.40 ± 37.23b	2333.58 ± 61.57	851.90 ± 32.62b	987.18 ± 45.87
Year						
2018	43	564.28 ± 13.23a	1355.03 ± 28.05a	2340.02 ± 46.38a	790.75 ± 24.57b	984.99 ± 34.55
2019	65	452.97 ± 11.61c	1132.15 ± 24.62b	2065.90 ± 40.72b	679.19 ± 21.57c	933.74 ± 30.33
2020	32	476.29 ± 12.89b	1405.80 ± 27.33c	2405.53 ± 45.19c	929.51 ± 23.94a	999.73 ± 33.67
Sex						
Female	119	455.90 ± 10.87B	1109.74 ± 23.05B	1918.01 ± 38.11B	653.78 ± 20.19B	808.28 ± 28.39B
Male	21	539.73 ± 16.16A	1485.58 ± 34.28A	2622.95 ± 56.68A	945.85 ± 30.03A	1137.37 ± 42.23A

LW 5 – live weight at 5 weeks of age; LW 12 – live weight at 12 weeks of age; LW 20 – live weight at 20 weeks of age; WG 1 – weight gain between 5 and 12 weeks of age, WG – weight gain between 12 and 20 weeks of age; $^{*}P < 0.05$ indicated with lowercase letters; $^{**}P < 0.01$ indicated with capital letters.

(501.19 ± 8.88 g) compared with homozygote animals TT (494.50 ± 17.56 g), but the difference was not significant. Later, at the age of 12 and 20 weeks, the homozygote animals TT had a higher live weight (LW 12 = 13.46 ± 37.23 g; LW 20 = 2333.58 ± 61.57 g) and weight gain (WG1 = 851.90 ± 32.62 g; WG 2 = 987.18 ± 45.87 g) compared with the heterozygote GT (LW 12 = 1248.92 ± 18.83 g, LW 20 = 2207.38 ± 31.14 g, WG1 = 747.73 ± 16.50 g; WG 2 = 958.46 ± 23.20 g). The animals with homozygote genotype TT at the age of 12 weeks were significantly heavier than the homozygote ones ($P < 0.05$). Also, the WG 1 between 5 and 12 weeks of age was significantly higher in homozygote animals TT ($P < 0.01$). The results demonstrated that the mutant allele T had a positive effect on body weight and weight gain. As shown in Table 2, we found a significant effect of the year on live weight and weight gain 1. We did not find a significant effect of the year on weight gain 2 from 12 to 20 weeks of age.

The obtained results also logically show a remarkably significant influence of sex on the monitored traits. Physiologically, roosters grow significantly faster and are heavier than hens in adulthood. When evaluating the effect of genotype on weight and weight gain by sex, females and males with genotype TT had a higher weight at 12 and 20 weeks of age, as well as weight gain. Females with genotype TT were heavier (1111.33 ± 44.96 g) than GT females (1108.15 ± 13.17 g). Similarly, rooster homozygotes were on average heavier (1581.47 ± 59.11 g) than heterozygotes (1389.69 ± 34.40 g). Both hens and roosters kept this trend at the age of 20 weeks. Homozygous TT animals achieved a higher average weight (hens: 1947.25 ± 74.35 g, roosters: 2719.91 ± 87.75 g) than heterozygous animals (hens: 1888.78 ± 21.78 g, roosters: 2525.99 ± 56.89 g). Similarly, weight gain was higher in homozygous animals than in heterozygous ones. Significantly higher WG 1 was observed in the homozygote TT males (1038.19 ± 51.78 g) compared with heterozygote GT (853.52 ± 30.14 g) ($P < 0.05$).

Wang et al. (2009) in their study confirmed that the *MC4R* G923T genotypes were significantly associated with live weight (LW), carcass weight (CW) and leg muscle weight (LMW). The LW of GT chickens was notably higher than that of TT ($P \geqslant 0.05$). Kubota et al. (2019) detected the significant association between the *MC4R* genotype and body weight at 2, 4, and 10 weeks of age of Korat chicken (KRC). KRC is a slow-growing chicken generated by crossbreeding male Thai indigenous chickens (Leung Hang Khao, LHK) and the female Suranaree University of Technology breeder line. The MC4R genotype had a significant

effect on body weight at 2, 4, and 10 weeks of age ($P < 0.05$); however, they could not detect any association between the *MC4R* genotype and the muscle fibre diameter.

In contrast to our results, where the higher live body weight was observed in the chicken with the TT genotype at 12 and 20 weeks of age, Molee et al. (2018) found the highest live body weight at 16 weeks of age in rapid growth rate chickens with the GG genotype (1478 ± 19.99 g). Chicken with genotype GT had LBW = 1408 ± 28.14 g, and chicken with genotype TT had LBW =1449 ± 13.19 g.

Conclusions

We can conclude that based on population screening and subsequent association studies of the *MC4R* gene polymorphism in relation to the observed production indicators of Oravka, we proved that after a long-time direction selection for breeding based on standard phenotype, preference is given to animals that are heterozygous (GT) in the *MC4R* gene, with a preference for the mutant allele frequency "T" = 54.61%. The homozygote animals TT had a significantly higher live weight and weight gain compared with the heterozygote GT, indicating that the mutant allele T has a positive effect on body weight and weight gain.

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