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The Single and Combined Effect of *MC4R* and *GH* Genes on Productive Traits of Pigs

[1]Lyubov Getmantseva, [1,2]Maria Leonova, [2]Aleksander Usatov, [1]Siroj Bakoev, [1]Aleksander Klimenko, [1]Vyacheslav Vasilenko, [1]Anatoly Kolosov, [1]Nadezhda Shirockova and [2]Maxim Makarenko

[1]*Don State Agrarian University, Persianovskiy, Russia*
[2]*Southern Federal University, Rostov-on-Don, Russia*

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Corresponding Author:
Lyubov Getmantseva
Don State Agrarian University,
Persianovskiy, Russia
Email: ilonaluba@mail.ru

Abstract: The aim of our work is to identify the single and combined effect of *MC4R* and *GH* polymorphism genes on growth and reproduction traits of pigs. The study was carried out on 204 crossbred F1 females (♀Landrace x ♂Large White) of the Breeding Farm in Russia. The analyzed productive traits were: The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA). The G1426A of *MC4R* and G316A of *GH* polymorphism were determined by PCR-RFLP method. The results showed a significant additive effect of *MC4R* (G1426A) on all analyzed traits and dominant effect on LB and NBA. With better Days to 100 kg associated genotype AA/*MC4R*, but the best indicators LB, BF and NBA installed for genotype GG/*MC4R*. The single effect of *GH* was determined to BF (dominant effect) and NBA (additive effect). The effect of genotype AG/*GH* on BF was observed in combination with genotypes of *MC4R*. In our studies we observed only six combinations of *MC4R* and *GH* genes instead of nine theoretically expected. The detected genotypes were following: AA/*MC4R* - AG/*GH*, AA/*MC4R* - GG/*GH*, AG/*MC4R* - AA/*GH*, AG/*MC4R* - AG/*GH*, AG/*MC4R* - GG/*GH*, GG/*MC4R* - GG/*GH*. The combination effect is defined for genotype AA/*MC4R* with genotypes of *GH*. The best means for all studied productivity traits were observed for the combination AA/*MC4R*-AG/*GH*. The combined effect of genotype AG/*MC4R* with genotypes of *GH* was not identified, but it was observed independently influence of *MC4R* on Days to 100 kg and NBA and *GH* on BF. The genotype GG/*MC4R* was detected only in combination with GG/*GH* genotype, which does not allow evaluating the combination effect of other *GH* genotypes.

Keywords: Combined Effect Genes, *MC4R*, *GH*, Pig, Growth, Reproduction

Introduction

The main direction of pig breeding is to provide new genetic knowledge about pig traits and focus on the identification and prediction of individual genes responsible for the reproduction, growth and meat traits (Fan *et al*., 2012; Yan *et al*., 2013; Leonova *et al*., 2015; Mihailov *et al*., 2014). Growth Hormone (*GH*), also known as somatotropin, is synthesized in the anterior pituitary (adenohypophysis in somatotropic cells). However, its production was found not only in the hypothalamus, but also in other parts of the nervous system and organs in the digestive tract. The *GH* for a long time been investigated as an anabolic hormone that regulates the metabolism, stimulates growth and overall body weight, promotes tissue regeneration and cell survival (Louveau and Gondret, 2004). Synthesis and secretion of *GH* is controlled somatoliberin hypothalamic hormones and somatostatin. Somatoliberin stimulates and somatostatin inhibits the secretion of *GH*, blocking the stimulating effect somatoliberin. The porcine *GH* gene was located on Chr12. A number of researches have showed the existence of an association between the polymorphism of *GH* gene and productive traits of pigs. There are data about *GH* gene polymorphism influence on conversion of the

feed, the number of Days to 100-kg, average daily gain, backfat thickness and others (Faria *et al*., 2006; Bižienė *et al*., 2011).

Adipose tissue is an active endocrine organ producing regulatory proteins (leptin, resistin, adipofilin, adipsin, agouti protein and others). Leptin is a major adipose tissue hormone, which regulates energy homeostasis, signaling the brain of fat reserves in the body. Melanocortin-4 Receptor (MC4R) is synthesized in the paraventricular nucleus of the hypothalamus and involved in leptin-induced signal transduction pathways (Huzar *et al*., 1997). The role of MC4R in feeding behavior and obesity implies that it may be significant genetic marker for growth traits of pigs. The porcine *MC4R* gene is located on Chr 1. Several studies have investigated the impact of *MC4R* gene polymorphism on growth and meat traits of pigs (Kim *et al*., 2000; Bruun *et al*., 2006; Klimenko *et al*., 2014; Van den Broeke *et al*., 2015). However, the main focus of the study is the effects of *MC4R* gene on growth and meat traits, but there are practically no data about influence of *MC4R* gene polymorphisms on reproductive traits of pigs. Also the scientific data cover only single effects of *MC4R* or *GH* gene on productive traits and there lack of information about combined impact of both genes. In connection with it, the aim of our work was to identify the single and combined effect of *MC4R* (G1426A) and *GH* (G316A) genes polymorphism on growth and reproduction traits of pigs.

Materials and Methods

Animals

The study was carried out on a cross F1 female (n = 204) to ♀Landrace x ♂Large White on the Breeding Farm in Russia. The farm specializes in breeding purebred pigs Landrace, Large White, Duroc and crossbred F1 (Landrace x Large White), F2 (F1xDuroc).

Studied Traits

The following productivity traits were measured: The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA). The traits of LB and BF were obtained according to the results of growing up to 100 kg. The NBA was obtained of the three farrowing. All pigs were kept under identical and standard conditions.

Genotyping

Extraction and subsequent analysis of porcine genomic DNA were performed in the Laboratory of molecular diagnostics and biotechnology Don State Agrarian University. DNA was isolated from whole blood samples using a Diatom DNA Prep100 (Isogene Lab. Ltd, Russia). The *MC4R (*G1426A) and *GH (*G316A) polymorphism were determined by PCR-RFLP method. For the PCR we used specific oligonucleotide primers (Kim *et al*., 2000; Faria *et al*., 2006). Restriction analysis of fragments amplified *MC4R* and *GH* were performed using restriction enzymes TaqI and FokI, respectively. Fragments were separated on a 3% agarose gel.

Statistical Analysis

Analysis of gene effect in the observed traits was examined using the following model:

$$Yijk = \mu + MC4Ri + GHj + G(MC4RxGH)ij + Sk + eijk$$

where: Yijkl –the observed trait (The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA)); μ – overall mean; MC4Ri - the effect of genotype *MC4R* (i = AA, AG, GG); GHj - the effect of genotype *GH* (i = AA, AG, GG); G (MC4RxGH)ij – interaction between genotypes *MC4R* and *GH* (ij=AA/*MC4R*-AA/*GH*, AA/*MC4R*-AG/*GH*, AA/*MC4R*-GG/*GH*, AG/*MC4R*-AA/*GH*, AG/*MC4R*-AG/*GH*, AG/*MC4R*-GG/*GH*, GG/*MC4R*-AA/*GH*, GG/*MC4R*-AG/*GH*, GG/*MC4R*-GG/*GH)*; Sk - effect of sire; eijk – random error.

The additive and dominant effects were calculated for single genes *MC4R* and *GH* according to the formulas proposed by Falconer and Mackay (1996). The effect of combined genotypes of *MC4R* and *GH* genes were calculated for following groups: (1) AA/*MC4R* with genotype of *GH* (AA/*MC4R*-AA/*GH*, AA/*MC4R*-AG/*GH*, AA/*MC4R*-GG/*GH*); (2) AG/*MC4R* with genotype of *GH* (AG/*MC4R*-AA/*GH*, AG/*MC4R*-AG/*GH*, AG/*MC4R*-GG/*GH*); (3) GG/*MC4R* with genotype of *GH* (GG/*MC4R*-AA/*GH*, GG/*MC4R*-AG/*GH*, GG/*MC4R*-GG/*GH*).

Results

The frequencies of single and combined genotypes of *MC4R* and *GH* genes are presented in Table 1. In the studied population of pigs were installed three genotypes of *MC4R* and three genotypes of *GH*, but the genotype AA/*GH* had a very low frequence.

In the first group identified two combination AA/*MC4R*-AG/*GH* and AA/*MC4R*-GG/*GH*; in the second group there were three possible combinations AG/*MC4R*-AA/*GH*, AG/*MC4R*-AG/*GH*, AG/*MC4R*-GG/*GH*; in the third group had only one combination GG/*MC4R*-GG/*GH*.

The single and combined effect genotypes of *MC4R* and *GH* genes on productive traits of crossbred F1 pigs are presented in Table 2.

Table 1. Frequencies of single and combined genotypes of *MC4R* and *GH* genes

Gene/Genotype (Frequency)	AA/*GH* (3.9)	AG/*GH* (41.2)	GG/*GH* (54.9)
AA/*MC4R* (21.5)	-	9.7	11.8
AG/*MC4R* (68.7)	3.9	31.5	33.3
GG/*MC4R* (9.8)	-	-	9.8

Table 2. The single and combined effect genotypes of MC4R and GH genes on productive traits of pigs

Genotype	Days of 100 kg	LB	BF	NBA
MC4R				
AA	154.92±0.86*[a]	122.79±0.37*[a]	13.42±0.05*[a]	12.14±0.21*[a]
AG	159.53±1.01	121.85±0.31[#]	13.10±0.06	13.10±0.19[#]
GG	161.41±1.24	124.10±0.42	12.65±0.03	13.23±0.20
GH				
AA	159.42±0.97	122.01±0.42	13.43±0.08	13.10±0.18*[a]
AG	158.14±0.89	121.17±0.48	12.58±0.07[#]	12.98±0.22
GG	157.96±0.99	122.96±0.39	13.35±0.04	12.65±0.16
MC4R - GH				
AA/MC4R - AG/GH	156.59±1.12*[1]	123.01±0.37	12.85±0.08*[1]	12.80±0.23*[1]
AA/MC4R - GG/GH	153.24±1.08	122.57±0.43	14.00±0.07	11.62±0.27
AG/MC4R - AA/GH	159.42±1.15	122.01±0.39	13.43±0.09	13.10±0.31
AG/MC4R - AG/GH	159.72±1.27	121.33±0.52	12.46±0.05*[2]	13.16±0.28
AG/MC4R - GG/GH	159.24±1.07	122.23±0.51	13.41±0.04	13.06±0.18
GG/MC4R - GG/GH	161.41±1.24	124.10±0.42	12.65±0.03	13.23±0.20

* - p≤0.05; [a]- additive effect; [#]- dominant effect; [1]- the effect of combined genotypes of MC4R and GH for first group; [2]- for second group

Days of 100 kg

Significant impact on the Days of 100 kg is defined only for genotype *MC4R*. Individuals of genotype AA/*MC4R* had better Days of 100 kg on 6.5 days (4.1%) relative to the genotype GG/*MC4R*. Single effect *GH* for Days of 100 kg is not established. However, when combined genotype AA/*MC4R* with genotypes of *GH* significant differences were established. Pigs with combination AA/*MC4R*-GG/*GH* had better Days of 100 kg on 3.4 days (2.2%) compared to the AA/*MC4R*-AG/*GH*. Nevertheless, the combination of genotype AG/*MC4R* with genotypes of *GH* showed no significant differences. In the third group, all individuals have a combination of genotype GG/*MC4R* - GG/*GH* and the lowest means of Days of 100 kg.

Length of Body

The single effect *MC4R* gene on LB was determined. Pigs of genotype GG/*MC4R* were longer on 1.6 cm (1.3%) compared genotype AA/*MC4R*. It was also determined that pigs heterozygous genotype AG/*MC4R* had smaller LB on 1.3 cm (1.1%) compared to homozygous. The significant single effect of *GH* gene on LB undefined. The combination of genotype AG/*MC4R* with genotypes of *GH* (AA, AG and GG) was observed at the smallest length for AG/*MC4R*-AG/*GH*, but the differences were not significant. The largest LB determined for genotype GG/*MC4R*.

Backfat Thickness

The significant single effects on BF were attributed for *MC4R* and *GH* genes. The minimum mean of BF was established for individuals of genotype GG/*MC4*R and AG/*GH*. Pigs of genotype GG/*MC4R* were lower BF on 0.77 mm (6.1%) compared to AA/*MC4R*. The BF for pigs of heterozygous genotype AG/*GH* was lower on 0.81 mm (6.4%) relative to homozygous of *GH*. At the same time the effect of genotype AG/*GH* clearly observed when combined with genotype AA/*MC4R* and AG/*MC4R*. The effect of genotype AG/GH, associated with less BF, in the first group for a combination of AA/*MC4R*-AG/*GH* and the second group of AG/*MC4R*-AG/*GH* were 1.15 mm (8.2%) and 0.96 mm (7.7%), respectively.

Number of Piglets Born Alive

Polymorphisms of *MC4R* and *GH* genes had significant single gene effects on NBA. Pigs of genotype GG/MC4R were more NBA on 1.0 piglet (7.3%) compared to the AA/*MC4R* and heterozygous AG/MC4R was significantly higher than homozygotes on 0.5 (3.5%). Also significant effect on the NBA has been set for the genotypes of *GH*. With the best mean of NBA associated genotype AA/GH (+0.5). The lowest NBA was genotype AA/*MC4R*. However, when combined genotype AA/*MC4R* with genotypes AG/*GH* and GG/GH identified significant differences. The combined AA/*MC4R*-AG/*GH* was NBA higher on 1.2 (10.1%) compared to the AA/*MC4R*-GG/*GH*. No significant differences in NBA combined genotype AG/*MC4R* with genotypes of *GH* were observed.

Discussion

The conducted research allowed identifying the single and combined effect of *MC4R* and *GH* genes on Days to 100 kg, LB, BF and NBA.

The results showed a significant additive effect of genotypes *MC4R* gene on all analyzed traits and the dominant effect on LB and NBA. Genotype AA/*MC4R* was associated with better Days to 100 kg, but more efficient values of LB, BF and NBA were observed in GG/*MC4R* genotype. In our study also established the positive effect of genotype GG/*MC4R* on the NBA mean. The significant single effect of *GH* on BF (dominant) and NBA (additive) was detected. It is interesting to note that the effect of AG/*GH* genotype on BF also was determined in combination with genotypes of *MC4R*.

Previously research on pure-bred Landrace pigs showed a positive effect of AG/*MC4R* on Days to 100 kg and crossbred pigs of GG/*MC4R* had increased BF values (Klimenko *et al*., 2014). Most previous data have shown that A allelic variant of *MC4R* is associated with best value of Days to 100 kg, however G allele and especially GG/*MC4R* genotype is associated with improved BF and meat traits (Dvořáková *et al*., 2011; Munoz *et al*., 2011; Van den Broeke *et al*., 2015).

However, there are own peculiarities for each population. This may be due to different combinations of genes and their effects. The analysis of multiple genes (LEP, LEPR, *MC4R*, PIK3C3 and VRTN) effects on production traits in Duroc pigs showed independently influence on growth rate and fat deposition (Hirose *et al*., 2014). We studied the combined effect of the *MC4R* and *GH* genes. In our population of nine possible genotypes were present only six: AA/*MC4R*-AG/*GH*, AA/*MC4R*-GG/*GH*, AG/*MC4R*-AA/*GH*, AG/*MC4R*-AG/*GH*, AG/*MC4R*-GG/*GH*, GG/*MC4R*-GG/*GH.*

The combination effect was observed between AA/*MC4R* and genotypes of *GH*. The best indicators for all four studied productivity traits were achieved with the combination AA/*MC4R*-AG/*GH*. The combined effect for genotype AG/*MC4R* with genotypes of *GH* was not determined and only independent influences of *MC4R* genotypes on Days to 100 kg, NBA and *GH* on BF were identified. To evaluate the stability of the combined effect between AA/*MC4R* and different allelic variants of *GH*, as well as finding other informative combinations further investigations need to be conducted on other breeds and crosses.

Conclusion

Our study has demonstrated the potential effect of the *MC4R* and *GH* genes and their different allelic combinations on growth and reproduction traits of crossbred pigs. We suggest that *MC4R* (G1426A) and *GH* (G316A) polymorphisms may be used in selection programs to improve economical traits in pigs.

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Author's Contributions

All authors equally contributed in this work.

Lyubov Getmantseva and Maria Leonova: Designed and performed experiments and wrote the paper.

Siroj Bakoev, Aleksander Usatov and Anatoly Kolosov: Developed analytical tools and analysed data.

Vyacheslav Vasilenko and Aleksander Klimenko: Designed and performed experiments.

Nadezhda Shirockova and Maxim Makarenko: Collected and analyzed data.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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