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Genetic Variability of Biochemical Blood Indicators and Economically Useful Traits of Young Hybrid Pigs of the Russian Population

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Abstract

The intensive development of genetic aspects in animal husbandry is a priority task for breeders now. New traits of the breeding process, including genetic assessments, are important for increasing product quality and organism directional resistance for breeding populations. This work aimed to study the genetically based relationship between biochemical parameters and economically useful traits of hybrid pig blood using mathematical methods applied in modern animal husbandry. The studies were carried out on boars (n=18) obtained by three-breed crossing (Large White×Landrace×Duroc) and grown at Shauer automatic feeding stations (Cooper, France) at Russian Hybrid Breeding Center. The fattening, meat (BW0, BW1, FG, ADG, P2), physical-chemical (moisture, protein, fat, ash, pH1, pH24) and biochemical (total protein, albumins, globulins, urea, creatinine, glucose, bilirubin, triglycerides, phospholipids, cholesterol, ALT, AST, ALP, calcium, phosphorus, magnesium, iron, chlorides) indicators were obtained. Automatic biochemical analyzer ChemWell (Awareness Technology, USA) and reagents from Analyticon Biotechnologies (Germany) and Spinreact (Spain) are used for determining biochemical indicators. The calculations and visualization of genetic and phenotypic correlations were carried out in R-studio. The obtained results will make it possible to increase the level of breeding research in the breeding population of animals that are used to obtain commercial hybrids.

Disciplinary: Agricultural Science, Bioscience.

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1 Introduction

The preparation of high-quality (including functional) food products and the development of genetic technologies are priority directions and guidelines for the development programs of the agro-industrial complex of the Russian Federation [1]. To increase the yield of "high-quality" and "cost-effective" livestock products, it is worth focusing on the research and technology development in the field of pig breeding, as one of the fastest-growing and promising branches of modern animal husbandry [2]. Of particular importance, both in biomedical and veterinary research directions, as well as in industrial farming is the study of hematological and biochemical parameters of blood in farm animals [3,4]. The biochemical composition of animal blood is influenced by age [5, 6], gender, breed [7], feeding conditions [8, 9] and the direction of productivity [10, 11], the season of the year [12, 13]. In particular, it was shown that the biochemical composition of the blood plasma of pigs is greatly influenced by the breed affiliation of individuals [7, 14]. This is especially evident when comparing the metabolism of lard pig breeds [10,11] with hybrids of lard and beef breeds [11]. Increased lipogenesis, reduced conversion of the nitrogen of amino acids to urea, as well as reduced protein synthesis, lead to increased fat deposition and accumulation of adipose tissue in lard pigs [10]. Whereas for hybrids (such as Duroc×Landrace×Large Yorkshire), the study by He et al. [11] showed that most of the feed energy is used for protein synthesis in skeletal muscles [11]. Particular differences in the biochemical composition of animal blood serum were also noted [3-7, 11]. For the obese pigs of the Ningxiang breed [11], the relatively high concentrations of insulin, some lipids (lipoproteins, triglycerides, unsaturated fatty acids), glycoproteins and Myo-inositol (i.e. "meso-Inositol"), as well as low concentrations of glucose (5.78±0.92 mmol/l), found in their blood serum as compared to hybrid animals (8.07±1.41 mmol/l) [11]. Madeira et al. [15] in their studies compared the biochemical parameters of the blood of two genotypes (so-called meat and sebaceous pig). The results of this research [15] show that lower plasma urea is one of the markers of the effective growth of lean tissue. Calculated Pearson correlations between plasma urea excretion and body nitrogen balance confirmed that low plasma urea concentration is indicative of higher efficiency of nitrogen utilization for lean-growing pigs [15].

The work carried out by Stevančević et al. [7] and Zahan et al. [16] on the study of meat productivity and biochemical blood parameters revealed a significant effect on the parameters of the age, breed and genotype of the animal. Palova et al. [17] noted that the levels of glucose, urea, gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), Ca and P were the highest in piglet blood at weaning compared to sires and sows [17]. Abeni et al. [18], when comparing clinical indicators in the blood of crossbred (Italian Duroc x Italian Large White) pigs and Duroc breed pigs, showed that the content of leukocytes, monocytes and lymphocytes in the group of crossbreed animals was higher than in the group of purebred pigs [18]. Some authors found a strong dependence on biochemical parameters by the animal genotype [19]. Erimbetov and co-authors (20) experimentally obtained positive correlations ($p<0.05$) of meat productivity (live weight, pulp and muscle mass output) with the activity of creatine-kinase and creatinine concentration in the blood, as well as a negative correlation ($p<0.05$) with the activity of the alkaline phosphatases in the pig blood [20]. Lingaas et al. [21] conducted a study on the heritability of 20 biochemical and immunological blood parameters based on samples taken from pigs undergoing performance testing at a station owned by the Norwegian Pig Association [21]. The need to determine genetic correlations between biochemical blood tests and other markers of productivity and health was also shown in the work of other researchers [22, 23].

In connection with the abovementioned relevance, the purpose of this work was to study the genetically based relationship between biochemical parameters and economically useful traits of hybrid boars, obtained by three-breed crossing (Large White×Landrace×Duroc), using mathematical methods applied in modern animal husbandry.

2 Materials and Methods

The studies were carried out on boars (n=18) obtained by three-breed crossing (Large White×Landrace×Duroc) and grown at Shauer automatic feeding stations (Cooper, France) at Russian Hybrid Breeding Center. The fattening, meat (BW_0, BW_1, FG, ADG, P2), physical-chemical (moisture, protein, fat, ash, pH_1, pH_{24}) and biochemical (total protein (TP), albumins (ALB), globulins (Glob), urea, creatinine (Creatin), glucose (Gluc), total bilirubin (NB), triglycerides (Trig), phospholipids (Phosp), cholesterol (Chol), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, phosphorus, magnesium, iron, chlorides) indicators were obtained.

Animals were bled from the jugular vein using a vacuum system (Vacuette) into test tubes for clinical trials (GreinerBio-One, Austria).

Automatic biochemical analyzer ChemWell (Awareness Technology, USA) and reagents from Analyticon Biotechnologies (Germany) and Spinreact (Spain) are used for determining biochemical indicators. The following methods were used:

biuret method – for total protein content,

bromcresol green method - for albumin content,

enzymatic colorimetric (according to Berthelot) method – for urea content,

kinetic methods – for creatinine content and activity of ALT, AST and ALT,

enzymatic-colorimetric methods –for the contents of cholesterol, triglycerides, phospholipids,

colorimetric methods – for the content of bilirubin, glucose, inorganic cations and anions.

Globulins were determined by the calculation method (subtracting albumins from the total protein) and the A/G ratio - by the division method.

The calculation of genetic and phenotypic correlations was carried out according to

$$r_g = \frac{r_{y1x2}}{2\sqrt{r_{x1x2} * r_{y1y2}}} \quad (1),$$

where: r_{y1x2}, r_{x1x2} and r_{y1y2} are coefficients of pair correlation of signs of descendants and ancestors.

Visualization of the results of phenotypic and genetic correlations was carried out in "R-studio" program using the "corrplot package".

3 Results and Discussions

The studies carried out on boars obtained by three-breed crossing (LW×L×D) under the following conditions (for the entire population): age of setting at feeding stations was 74 days (live weight was about 37 kg), the age of removal was 151 days (live weight was about 111 kg), the fattening period was about 77 days, the total gain was about 74 kg. All these conditions correspond to the regulatory and technological criteria for automatic feeding stations. The average daily gain was about 960 g/day, and the thickness of the fat between the 6th-7th thoracic vertebra was about 21 mm. The physicochemical parameters of meat and the biochemical parameters of the blood of the studied population are within the reference values [24, 25, 26] and have a coefficient of variability from 1.4% (low) to 44.0% (high), which indicates the heterogeneity of indicators across the population hybrid pigs.

It should be noted that both the physicochemical parameters of meat and the biochemical parameters of blood related to proteins and determined experimentally have a fairly low coefficient of variability: protein in meat (Prot) - 4.0%; TP - 7.8%; A - 10.9%. On the other hand, similar parameters of meat and blood related to fats (lipids), carbohydrates, enzymes and a number of other BAS have a coefficient of variability 5-10 times higher than protein ones. This can serve as an indicator of the advanced growth of protein-muscle mass with stable protein metabolism, which plays an important role in the intensive fattening of individuals. It should be noted that all experimentally determined values of both the physicochemical parameters of meat and the biochemical parameters of blood for commercial hybrids are within the physiological and biochemical norms of healthy animals for each of the above breeds [27, 28].

To study the dependence of fattening parameters and the biochemical composition of the blood, a regression analysis was carried out (Figure 1), which revealed a significant relationship between the initial live weight and total bilirubin in the blood (R^2 = 39.6%) and the final live weight with the content of creatinine in the blood (R^2=21.3%).

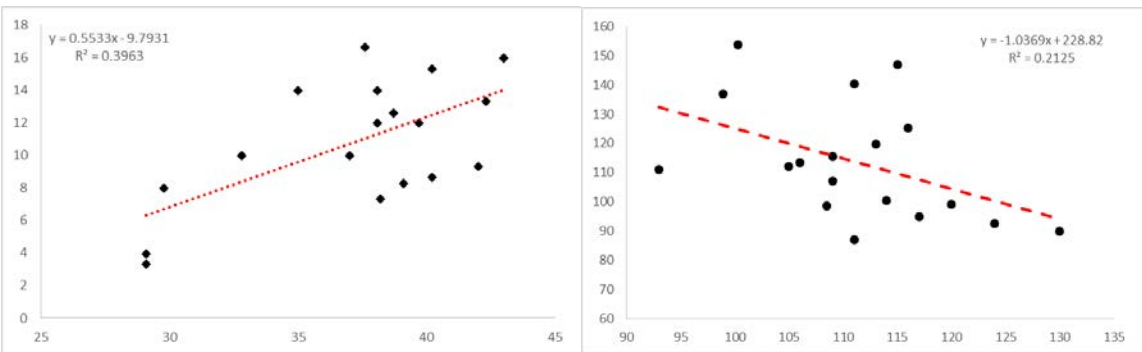

Figure 1: Linear relationship between indicators: a) initial live weight and the content of total bilirubin in the blood; b) final body weight and blood creatinine.

The determination of the linear dependence of traits is a universal form of research for the subsequent identification of the presence of relationships in the form of genetic and phenotypic correlations of the studied traits. Since a linear relationship has been established precisely with the initial and final live weight of the studied three-breed pig hybrids, so, bilirubin and creatinine can be considered as indicators associated with the processes of gaining live weight and directly related to diets. Bilirubin as an end product of the breakdown of hemoglobin, myoglobin and cytochrome may indicate the intensity of processes associated with cellular respiration and high energy costs associated with gaining muscle mass during the fattening period. The level of creatine is the end product of the creatine-phosphate reaction of energy metabolism in muscle tissue.

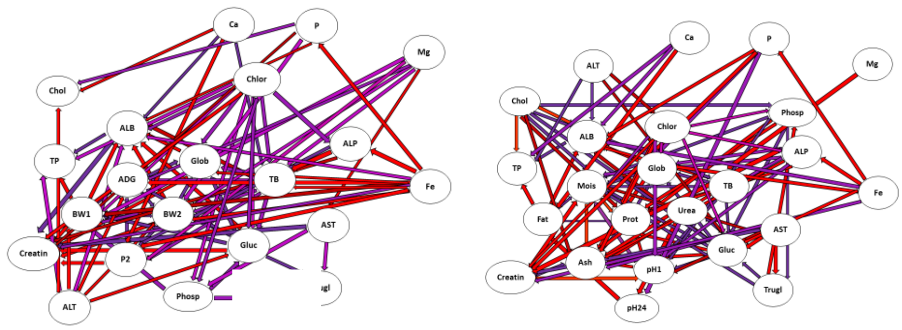

Figure 2: Network of phenotypic correlations ($r \geq 0.50$) between: a) fattening parameters and blood biochemical parameters; b) physical and chemical indicators and biochemical parameters of the blood of crossbred pigs. Red lines indicate negative correlated traits and violet lines indicate positive correlations. **Notes:** *BW_1 – live weight when placed at feeding stations, kg; BW_2 - live weight when removed from feeding stations, kg; ADG – average daily gain, g/day; P2 is the thickness of the bacon between the 6th-7th thoracic vertebrae, mm; Mois - moisture,%; Prot—protein, %; Fat - fat,%; Ash - ash, %; pH_1 - acidity indicator (45-60 minutes after slaughter); pH_{24} - acidity indicator (16-28 hours after slaughter); TP—total protein, g/l; ALB—albumin, g/L; Glob—globulin, g/l; Urea—urea, mM/l; Creatin—creatinine, µM/L; Gluc—glucose, mM/l; TB—total bilirubin, µM/l; Trigl—triglycerides, mM/l; Phosp—phospholipids, mM/l; Chol—cholesterol, mM/l; ALT—alanine aminotransferase, IU/l; AST—aspartate aminotransferase, IU/L; ALP—alkaline phosphatase, IU/L; Ca—calcium, mM/l; P—phosphorus, mM/l; Mg—magnesium, mM/l; Fe—iron, mM/l; Chlor – chlorides, mM/l.*

The results (Figure 2) show that all of the identified pairs of combinations of phenotypic values, and the estimates of the relationship of the studied animals according to fattening traits indicate a high level of relationships between these animal traits ($p \leqslant 0.001$). At the same time, a large percentage of positive values (57.6% and 55.8%) was revealed among genetic relationships and among phenotypic ones (respectively). The highest values of positive phenotypic relationship were established between the following traits: fat thickness between the 6th-7th thoracic vertebra and: concentration of iron (Fe) -0.67; calcium content (Ca) -0.63; average daily gain and: globulin concentration +0.68, creatinine concentration -0.74, glucose concentration +0.57 and iron (Fe) concentration -0.55; as well as live weight by removing from feed stations and: albumin concentration -0.57, globulin concentration +0.67, creatinine concentration -0.70, total bilirubin concentration +0.63; ALT activity -0.57 and iron (Fe) concentration -0.64. Important are the relationships between: live weight at fattening and total protein -0.50, the amount of albumin -0.57, creatinine -0.56, glucose 0.56, total bilirubin 0.70, phospholipids 0.59, chlorides 0 .67, ALT -

0.54 and trace elements: magnesium 0.53, iron -0.52. According to Physico-chemical indicators, significant relationships were established between moisture with protein, ash and pH1, which amounted to -0.69, -0.90 and 0.67, respectively. Phenotypic correlations with the biochemical composition of blood were as follows: moisture with globulin, creatinine, total bilirubin, triglycerides, phospholipids, cholesterol, AST, alkaline phosphatase and chlorides 0.56, -0.56, 0.65, 0.55, 0.79, 0.53, -0.63, 0.50 and 0.69, respectively. Since the moisture content (which has an optimal value in the studied age group - 73.6%) in meat directly depends on the age, fatness and feeding of the animal (phenotypic factors), as well as changes in the biochemical composition of the blood, the reliability of the information received has a scientific justification. Relationships between the other parameters (fat, ash, protein) and meat acidity (pH1, pH24) also have strong correlations shown in Figure 3b.

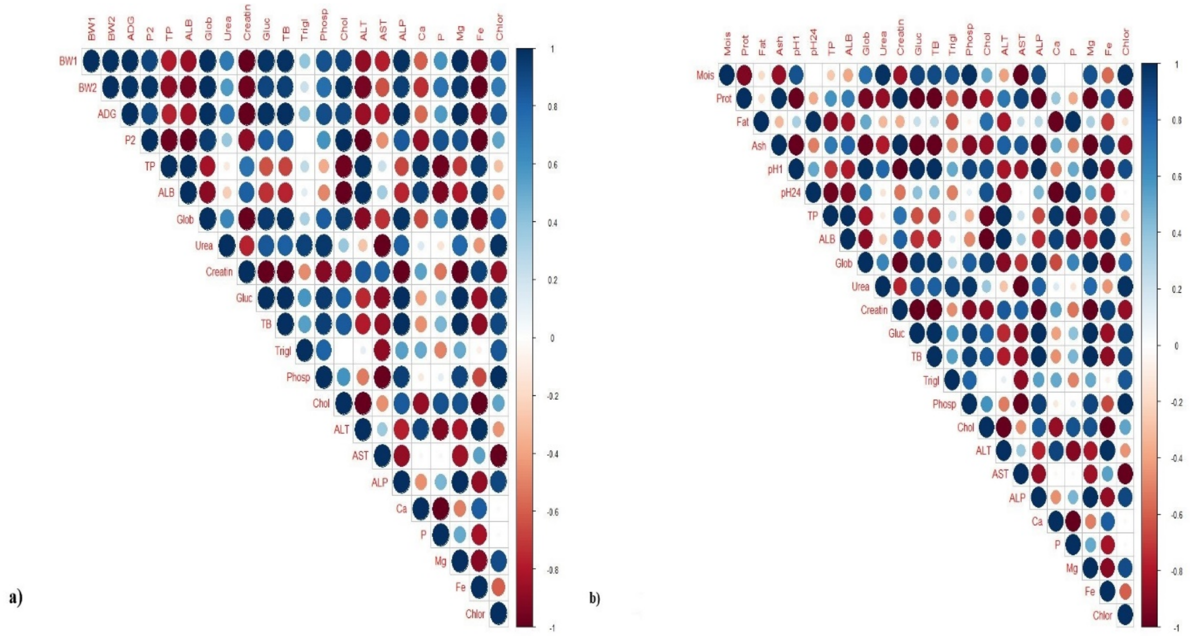

Figure 3: Heat map of genetically based correlations estimated between a) fattening parameters and blood biochemical parameters; b) physical-chemical indicators and blood biochemical parameters of the crossbred pigs. **Notes:** *BW_1 – live weight when placed at feeding stations, kg; BW_2 - live weight when removed from feeding stations, kg; ADG – average daily gain, g/day; P2 is the thickness of the bacon between the 6th-7th thoracic vertebrae, mm; Mois - moisture,%; Prot—protein, %; Fat - fat,%; Ash - ash, %; pH_1 - acidity indicator (45-60 minutes after slaughter); pH_{24} - acidity indicator (16-28 hours after slaughter); TP—total protein, g/l; ALB—albumin, g/L; Glob—globulin, g/l; Urea—urea, mM/l; Creatin—creatinine, µM/L; Gluc—glucose, mM/l; TB—total bilirubin, µM/l; Trigl—triglycerides, mM/l; Phosp—phospholipids, mM/l; Chol—cholesterol, mM/l; ALT—alanine aminotransferase, IU/l; AST—aspartate aminotransferase, IU/L; ALP—alkaline phosphatase, IU/L; Ca—calcium, mM/l; P—phosphorus, mM/l; Mg—magnesium, mM/l; Fe—iron, mM/l; Chlor – chlorides, mM/l.*

To visualize the obtained data of genetically based relationships, a "heat map" was created (Fig. 3), which clearly shows the dependence of the studied traits on the genotype of the parents. Live weight at fattening start directly depends on live weight at removal from fattening (r_g = 0.64) and the average daily gain positively correlates with live weight at r_g = 0.54 and 0.96, respectively. Considering the biochemical components of the animal blood, a strong relationship was found between the live weight at the fattening start and the total protein of 0.63. Between the physical-chemical parameters of meat and blood biochemistry (Fig. 3b), high genetically based correlations were found between moisture with glucose and phospholipids (r_g = 0.57 and 0.54); fat correlates negatively with total protein and globulins (r_g = -0.50 and -0.53, respectively). Ash is significantly correlated with glucose and total bilirubin (r_g = -0.53 and -0.51), while the acidic composition of meat revealed a genetically based correlation between pH_{24} and urea (r_g = -0.56), which is explained by the concentration of nitrogen-containing elements and urea what can cause the manifestation of the syndrome of "sour" meat.

The high degree of correlation between the concentration of phospholipids and triglycerides and glucose is explained by the “co-participation” (“mutual involvement”) of these metabolites in carbohydrate-lipid metabolism in the body. First of all, in terms of their functions in the body, both triglycerides and carbohydrates are used as energy sources for metabolic processes. A lot of lipids (phospholipids, cholesterol, triglycerides, etc.) together with proteins participate in the formation of cell membranes, chylomicrons and various lipoproteins, etc. [11,12].

4 Conclusion

This work was carried out using genome-wide association studies Genome-Wide Association Studies (GWAS), which are necessary to study the genetic aspects of meat and biochemical parameters of commercial hybrids. The studied biochemical parameters of boar’s blood obtained by three-breed crossing (LW×L×D) are within the species reference values both for the beginning and for the end of the fattening period. Our data confirm the strict regulation of biochemical homeostasis, which is preserved in the process of genetic variability during crossbreeding involving three breeds of pigs. High phenotypic correlations were obtained between fattening, the physical-chemical composition of meat and blood biochemistry, which indicate and confirm a direct dependence on the conditions of keeping and feeding individuals. The revealed high genetically based correlations confirm the dependence of obtaining a quality meat yield on breeding parents.

When modeling a linear relationship that reflects complex relationships between phenotypic and biochemical characteristics, indicators such as bilirubin and creatinine can be considered not only in the framework of classical biochemistry as indicators of liver function (bilirubin) and renal filtration (creatinine) but also as indicators associated with the intensity live weight gain. For an indirect assessment of the intensity of processes associated with oxygen transfer and cellular respiration, as well as energy costs associated with gaining muscle mass during the fattening period. The results obtained indicate the correct direction of the ongoing work and, in the future, the study of the biochemical components of blood in the genetic context, due to which the level of breeding work in the breeding population of animals used to obtain commercial hybrids will increase, taking into account all factors.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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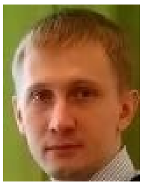

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