

## RESEARCH ARTICLE

## COMPARATIVE ANALYSIS OF AMINO ACID CONTENTS OF HYBRID ANIMALS AT SETTING UP AND REMOVAL FROM FATTENING

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## ARTICLE DETAILS

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## ABSTRACT

The contents of amino acids (AAs) in the major tissues of living creatures are currently being actively studied in the world, because AAs are between the most important "biologically active substances" (BAC). These BAC are not only construction elements of the body but also valuable products for animal and human nutrition. The aim was to identify patterns in the AA-content of the hybrid pig blood during feeding and removal from fattening, including correlations both between AAs and with the key "biochemical values of animal blood" (BVAB). The high-performance liquid chromatography method was used to determine the AA-content of the blood of the hybrids (Large White × Landrace × Duroc) that is between the key indicators for monitoring of the pig product quality. Statistical processing is performed in Microsoft Excel. The data distribution corresponds to a normal distribution, "correlation coefficients according to the Pearson method" (CCPM). The drawings are in the R program. For the first time, the CCPM of amino acids among themselves and with the BVAB were obtained. These values for all the studied animals are significantly higher after fattening ( $r=0.60-0.88$ ) than in the initial period of fattening ( $r=0.17-0.35$ ). The obtained CCPM make it possible to reduce the data array for further search and study of significant blood parameters to improve the efficiency of fattening. The most promising candidates for this are CCPM between amino acids and proteins as key BVAB.

## KEYWORDS

pig hybrids, blood, meat, amino acids, correlations.

## 1. INTRODUCTION

The meat production and consumption is growing constantly in all countries in the world leading to the intensification of livestock farming (Mateos et al., 2024; Kim et al., 2024; Ahmad et al., 2018; FAO, 2025; USDA, 2024; Barbut, 2020). The present state in this field in the world is briefly summarized in the FAO documents such as "Biannual report on global food markets. Food Outlook, November 2025" (FAO, 2025). For example, in 2023, 2024 and by November 2025 (FAO, 2025) the total meat production (including pig meat) in the world was about 372.2 (124.7) 378.1 (125.1) and 383.5 (126.3) million tonnes (carcass weight equivalent) (FAO, 2025). These points are also important for description of the meat market in Russia during last decades (Zaitsev et al., 2021; Dankvert, 2021; Chinarov, 2017). Since 2019 Russia ranked at 4-5 places (together with Brazil) in terms of the number of pigs and pork production, followed by China, the European Union and the United States (Zaitsev et al., 2021; Dankvert, 2021; Chinarov, 2017).

In the recent years, pork has remained second in Russia (after poultry) both in terms of total meat production, as well as in terms of consumption by the population (Zaitsev et al., 2021; Dankvert, 2021; Chinarov, 2017). For example, in 2025, pork production in Russia amounted to 4.87 million tons in slaughter weight, which is almost the same as in 2024. Pork continues to account for a significant share of total meat production, in Russia amounting to 39.5% in 2025 (MEATINFO, 21.01.2026 <https://meatinfo.ru/news/proizvodstvo-svinini-v-rossii-tendentsii-2025-491017>). The total meat consumption by the population (in general)

in 2023, 2024 and by November 2025 (FAO, 2025) in the world was about 45.8 46.2 and 46.4 (kg/year per caput food consumption) (FAO, 2025).

Pork quality, i.e. its physicochemical and other properties, depends on the AA-content of the hybrid pig (Leroy et al., 2023; Kamonpatana, 2019; Belitz et al., 2008). Muscle AA-content and proteins largely determine the hybrid pig worth, as well as the consistency, taste, color of meat. For example, muscle contains the highest amounts of glutamic acid and glutamine, which impart meaty flavor (Dalibard et al., 2014; Dołowy and Pyka, 2014; Karau and Grayson, 2014; Wu et al., 2010).

Various chromatographic methods (HPLC) are currently widely used for the analysis of biologically active substances, including amino acids (Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020; Erimbetov, 2018). Post-column derivatized HPLC is the most suitable for the quantitative monitoring of AA-content in biological samples. The main advantages of this method are high determination accuracy, reproducibility, and reliability, as well as simplified sample preparation (compared to pre-column derivatization methods) (Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020).

Determining the amino acid composition of proteins is associated with enormous biological significance, particularly for L- $\alpha$ -amino acids, in human and animal life, as well as in nature in general (Dalibard et al., 2014; Dołowy and Pyka, 2014; Karau and Grayson, 2014). Amino acids perform a wide range of functions essential for the physiological-biochemical status: biosynthesis and protein-amino acid metabolism; precursors of several neurotransmitters and hormones that regulate the central nervous

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system, carbohydrate, lipid, and water-salt metabolism; maintenance of mental and brain activity; tissue regeneration; and muscle fiber formation (Zaitsev et al., 2022; Wu et al., 2010).

Some amino acids can directly supply energy to muscle tissue, suggesting that the key AA properties enable the body to grow, develop, and function normally throughout life, as they are essential for all vital processes (Zaitsev et al., 2022; Wu et al., 2010). Amino acids are key components of animal nutrition, both as part of a protein diet and as supplements (Dalibard et al., 2014; Dołowy and Pyka, 2014; Karau and Grayson, 2014; Wu et al., 2010; Belitz et al., 2008). In this regard, a huge number of different feed additives, original combinations, and feed formulations are being developed that contain the essential amino acids for balanced growth and development in pigs (Dalibard et al., 2014; Dołowy and Pyka, 2014; Karau and Grayson, 2014; Wu et al., 2010; Belitz et al., 2008). The objectives of our study were to identify patterns in the AA-content of the hybrid pig blood during introduction and weaning, including correlations (CCPMs) between AAs and with the key "biochemical values of animal blood" (BVAB).

## 2. MATERIALS AND METHODS

The sixty-eight crossbreed pigs: Large-White×Landrace×Duroc (LW×L×D) were selected for the study at the time of setting up and removal from fattening. The age at which the pigs were placed on automatic feeding stations for the entire population was 74 days, and the age at which they were exited was 151 days. The fattening period was 77 days, and the gain was approximately 74 kg, which meets the regulatory and technological criteria for the feeding stations. Analysis of fattening parameters revealed that the live weight at entry was 37.2 kg, while the average live weight at exit from fattening was approximately 111 kg, which meets the technical criteria for the feeding stations (Zaitsev et al., 2022).

Amino acid concentrations in blood serum were determined by post-column derivatized HPLC of blood samples. For this purpose, the "LC-20 Prominence high-performance liquid chromatography system" ("Shimadzu", Japan) modified by "Sevko&Co" (Russia) (Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020). Sample preparation for

analysis was made by GOST 32195-2013. Samples were hydrolyzed in an acidic medium (6N HCl). After hydrolysis, the samples were dried at 1000°C; dissolved and centrifuged at 13,000 rpm. The supernatant was analyzed for amino acids (Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020).

Since methionine and cysteine are destroyed by hydrolysis, they must first be converted to a stable form by oxidation to cysteine acid and methionine-sulfone. Tyrosine, on the other hand, is destroyed by oxidation followed by hydrolysis. Therefore, for a complete AA-analysis of the test sample, two samples are required: "one oxidized and one unoxidized" (Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020).

The detection limit for most amino acid derivatives is typically 10 pmol, and for proline derivatives, 50 pmol. Signal linearity is observed in the range of 20–500 pmol, with a correlation coefficient greater than 0.999. During the study, it was concluded that, to obtain satisfactory data, protein or peptide samples weighing greater than 1 µg should be used before hydrolysis (Zaitsev et al., 2022; Zaitsev et al., 2022; Zaitsev et al., 2021; Bogolyubova et al., 2020). Statistical processing is performed in Microsoft Excel. To characterize the central trend, the values of the average values and the standard error are presented, the distribution of the data obtained corresponds to a normal distribution, and the correlation coefficient was calculated using the Pearson method.

## 3. RESULTS AND DISCUSSION

Amino acid content is a key indicator of pork product quality. Amino acid analysis was performed on 68 blood samples from hybrid pigs. Since not all α-amino acids are readily quantifiable, chromatography primarily assesses the total content of amino acids such as Tyr, Phe, Gly, Ala, Val, Leu, Ile, Asp, Glu, Arg, His, Lys (at 570 nm), and Pro (at 440 nm). During hydrolysis (6M HCl, 24 hours, 110°C), Trp, Asn, and Gln are destroyed, while Cys and Met are easily oxidized. Additionally, Tyr, Ser, and Thr are partially lost and can be determined as a sum (as Tyr + Phe or Ser + Thr). Importantly, we determined the essential α-amino acids (after total hydrolysis of samples) in two groups: the first, at placement (42 samples) and the second, at weaning (26 samples) (Figure 1 and 2).

**Table 1:** Analysis of essential amino acids (g/100 ml) in the blood of hybrid pigs (setting up) (n=42, first group)

Amino acids	M±m, (g/100 ml)	SD, a.u.	Var., a.u.
Aspartic acid	0.55±0.01	0.04	0.02
Threonine	0.31±0.01	0.03	0.01
Serine	0.31±0.01	0.03	0.01
Glutamic acid	0.84±0.02	0.07	0.03
Glycine	0.20±0.01	0.02	0.01
Alaine	0.38±0.01	0.04	0.02
Valine	0.39±0.01	0.03	0.01
Isoleucine	0.23±0.01	0.02	0.01
Leucine	0.65±0.02	0.05	0.02
Tyrosine	0.34±0.01	0.03	0.01

**Table 1 (Cont):** Analysis of essential amino acids (g/100 ml) in the blood of hybrid pigs (setting up) (n=42, first group)

Phenylalanine	0.37±0.01	0.03	0.01
Histidine	0.21±0.01	0.02	0.01
Lysine	0.59±0.01	0.06	0.03
Arginine	0.37±0.01	0.03	0.01
Proline	0.27±0.01	0.02	0.01

**Table 2:** Analysis of essential amino acids (g/100 ml) in the blood of hybrid pigs (removal) (n=26, second group)

Amino acids	M±m, (g/100 ml)	SD, a.u.	Var., a.u.
Aspartic acid	0.67±0.02	0.12	0.06
Threonine	0.39±0.01	0.07	0.04
Serine	0.39±0.01	0.07	0.04
Glutamic acid	0.99±0.03	0.17	0.09
Glycine	0.24±0.01	0.04	0.02
Alaine	0.42±0.01	0.07	0.04
Valine	0.47±0.01	0.08	0.04
Isoleucine	0.26±0.01	0.05	0.02
Leucine	0.74±0.02	0.12	0.06
Tyrosine	0.40±0.01	0.07	0.04
Phenylalanine	0.43±0.01	0.08	0.04
Histidine	0.23±0.01	0.05	0.03
Lysine	0.66±0.02	0.11	0.06
Arginine	0.42±0.01	0.11	0.06
Proline	0.35±0.01	0.07	0.04

As a result of the analysis of the data in Table 1, all AA can be divided into three groups based on their content in the blood of pigs when put on fattening: the first - four AA, the content of which is higher than 0.5 g / 100

g (Glu> Leu> Lys> Asp), the second - seven AA, the content of which is from 0.3 to 0.5 g / 100 g (Val> Ala> Phe> Arg> Ser> Thr> Tyr), and the third - four AA, the content of which is less than 0.3 g / 100 g (Pro> Ile> His> Gly).

From the analysis of the data in Table 2 it follows that the same three groups of AA practically do not change in composition in the blood of hybrid pigs after weaning: firstly, four AA, the content of which is higher than 0.5 g/100 g (Glu>Leu>Asp>Lys), secondly, eight AA, the content of which is from 0.3 to 0.5 g/100 g (Val>Phe>Ala>Arg>Tyr>Thr>Ser>Pro), and thirdly, three AA, the content of which is less than 0.3 g/100 g (Ile>Gly>His). However, the absolute values of the content of these AAs in the blood of hybrid pigs after weaning (Table 2) are significantly higher than they were when putting them on fattening (Table 1): Asp (by 21.8%),

Thr (by 12.9%), Ser (by 12.9%), Glu (by 17.9%), Gly (by 20.0%), Ala (by 10.5%), Val (by 20.5%), Ile (by 13.0%), Leu (by 13.8%), Tyr (by 17.79%), Phe (by 16.2%), His (by 9.5%), Lys (by 11.9%), Arg (by 13.5%), Pro (by 29.6%). Thus, all AA can be arranged in a sequence based on their increased blood levels in hybrid pigs after weaning compared to when started fattening  
Pro>Asp>Val>Gly>Glu>Tyr>Phe>Arg>Leu>Ile>Ser=Thr>Lys>Ala>His. Correlation values for the blood levels of all studied  $\alpha$ -amino acids (among themselves) in hybrid pigs were determined (Figure 1 and 2).

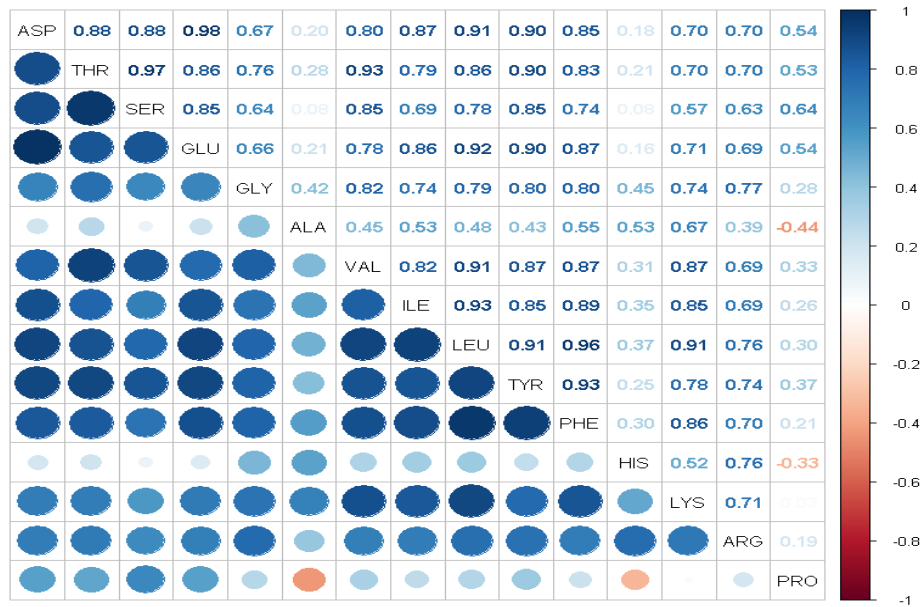


Figure 1: Correlation coefficients between the main AA in the blood of hybrid pigs (setting up at fattening)

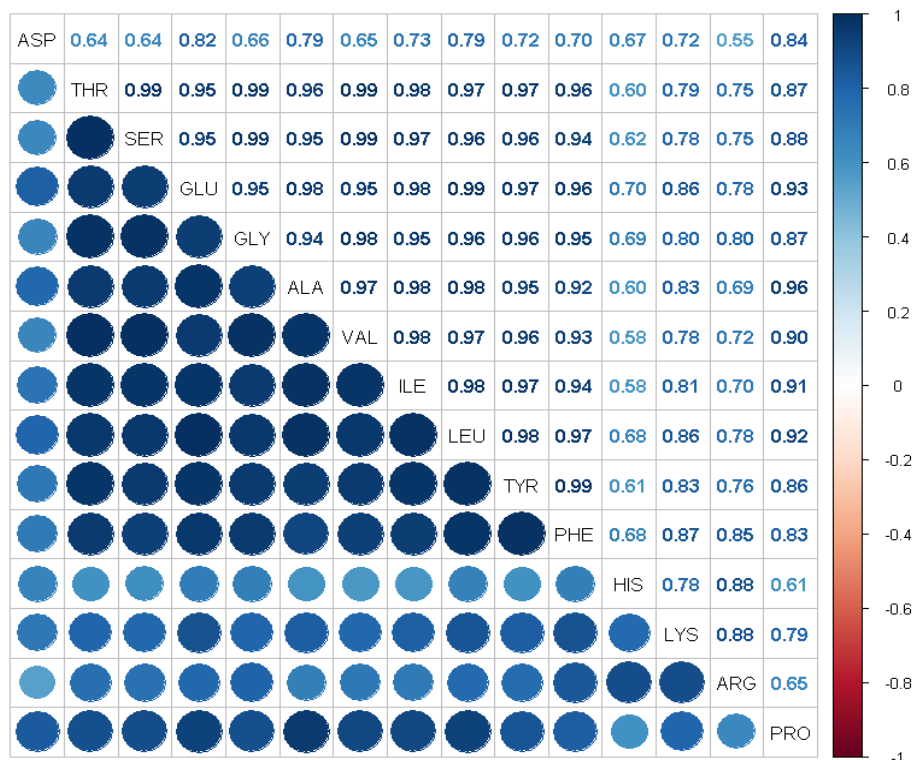


Figure 2: Correlation coefficients between the main AA in the blood of hybrid pigs (after removal from fattening)

Exceptionally high correlation values (ranging from 0.70 to 0.99) were found for the levels of all  $\alpha$ -L-amino acids studied in the blood of hybrids during by setting up at fattening (Figure 1) and after removal from fattening (Figure 2). These data provide direct evidence of the validity of our chosen research strategy and methods for analyzing the essential amino acid content in these samples.

In the first group of hybrids (setting up fattening), the following amount of correlation (very strong/strong/moderate) between specific amino acids was revealed in the blood: 8/4/0 – for Asp, 9/3/1 – for Thr, 7/5/0 – for

Ser, 8/4/0 – for Glu, 7/4/0 – for Gly, 0/4/7 – for Ala, 10/1/3 – for Val, 9/3/0 – for Ile, 11/0/3 – for Leu, 10/1/2 – for Tyr, 10/2/1 – for Phe, 1/2/6 – for His, 5/8/0 – for Lys, 3/9/1 – for Arg, 0/4/5 – for Pro (weak correlations, i.e. less than 0.25, are not considered).

In the second group of hybrids (after removal from fattening), the following amount of correlation (very strong/strong/moderate) between specific amino acids was found in the blood: 4/10/0 – for Asp, 12/2/0 – for Thr, 12/2/0 – for Ser, 13/1/0 – for Glu, 12/2/0 – for Gly, 12/2/0 – for Ala, 11/3/0 – for Val, 11/3/0 – for Ile, 13/1/0 – for Leu, 12/2/0 – for Tyr,

12/2/0 – for Phe, 2/12/0 – for His, 13/1/0 – for Lys, 9/5/0 – for Arg, 12/2/0 – for Pro (weak correlations, i.e., less than 0.25, are not considered).

Thus, the total number of very strong and strong correlations between specific AAs in the hybrid blood in the second group is greater than or equal to those in the first group of animals. As can be seen from Figures 1 and 2, some exceptions are the correlation values of alanine, histidine, and proline content in blood samples of the first group of hybrids, while the aspartate content is slightly reduced in blood samples of the second group of hybrids compared to other amino acids (but not below the average correlation values). Thus, all AAs maintain or increase correlation values (between specific AAs) in the blood of hybrids in the second group compared to those in the first group of animals. Strong correlations of AA-content in the hybrid pigs blood (at the time of weaning) were noted: with total protein (for eight amino acids  $r=0.50-0.64$ , six amino acids  $r=0.30-0.46$ ); albumin (for seven amino acids  $r=0.50-0.69$ , six amino acids  $r=0.30-0.46$ ); urea (for Pro  $r=-0.91$ , Glu, Ala  $r=-0.80$ , seven AA  $r=0.54-0.74$ , etc.); phospholipids (for nine AA  $r=0.50-0.65$ ); with a number of cations and chlorides (for many AA  $r=0.50-0.75$ ). This is explained by the peculiarities of the participation of certain AA in the main metabolisms.

As visualized in the correlation matrix, metabolic synchronization for the AAs we studied changes with age and is expressed to varying degrees for different AAs. With age, the direction and strength of the CCPMs between AAs with one another become stronger, with the direction of this CCPM being strictly positive for all AAs. Based on this, we can assume that an increasing number of AAs are involved in metabolism. Furthermore, we observe the following pattern: branched-chain AAs (Val, Ile, Leu) form a tight cluster with aromatic AAs (Tyr, Phe). Notably, the relationship between Tyr and Phe after fattening was nearly linear ( $r=0.99$ ,  $p<0.001$ ). This may reflect their common pathways of inclusion in metabolism or a simultaneous change in the scale of their inclusion in metabolism that is not synchronized across metabolic pathways. Furthermore, the strong correlation between glutamate and leucine ( $r=0.99$ ) highlights the key role of Glu as a nitrogen donor in transamination reactions during intense muscle growth.

#### 4. CONCLUSION

Thus, HPLC was used to determine the AA-content of the hybrid pig blood (Large White × Landrace × Duroc). For the first time, the correlation coefficients of amino acids among themselves and with the key biochemical values of animal blood were obtained. These values for all studied animals were significantly higher after removal from fattening ( $r=0.60-0.88$ ) than during the initial period before fattening ( $r=0.17-0.35$ ). The obtained correlation coefficients allow us to reduce the data set for further search and study of significant blood parameters to improve fattening efficiency. The most promising candidates for this purpose are correlations between amino acids and protein parameters in the blood.

#### AUTHORS CONTRIBUTIONS

SYZ, NSK, OAV participated in study design and planning, data measurements and analysis, and drafting of the first version of the document. VNT, AAM participated in surveys data collection and interpretation, reviewed the manuscript.

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