

Review

Chromatographic Methods for Evaluation of the Amino Acid and Fatty Acid Compositions Related with Pork Quality

Nadezhda V. Bogolyubova, Sergei Yu. Zaitsev *

L.K. Ernst Federal Science Center for Animal Husbandry, Dubrovitsy 60, Podolsk Municipal District, 142132 Moscow, Russia; 652202@mail.ru (N.V.B.); s.y.zaitsev@mail.ru (S.Yu.Z.).

* Correspondence: s.y.zaitsev@mail.ru (S.Yu.Z.); Tel.: +7-4967-651363

Abstract: The increasing demand for high-quality livestock products dictates to develop approaches to assessing the composition of the fatty acids (CFAs) and amino acids (CAAs) in animal tissues. The review considers the following issues: chromatographic methods for the determination of CAAs and CFAs of pig tissues; factors influencing the CAAs and CFAs of pig tissues; methods of regulating CAAs and CFAs of pork using nutritional factors; the effect of CAAs and CFAs on formation of meat properties. The main methods for determining CAAs or CFAs are the ion-exchange or gas chromatography, respectively. The total FA amount and individual FAs have significant effects on the tenderness, taste, color and juiciness of pork meat (due to the different melting points of particular fatty acids, formation of lipid oxidation products during cooking, etc.). Muscle proteins of pigs with regulated fatness differ also in CAAs (decreasing by increase in “pork fat” and decrease in the protein’s amount. The significance of this review is also determined by high popularity of pork in Russia and in a number of other countries of the world.

Keywords: chromatography, amino acids, fatty acids, pork analysis, adipose tissue, muscle tissue, nutritional factors.

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Abbreviations:

AAs - amino acids,
ATP - adenosine triphosphate,
BACs - biologically active components,
BJBP - Berkshire × JBP,
DBJBP - Duroc × Berkshire × JBP,
DLJBP - Duroc × Landrace × JBP,
DIAAS - digestible indispensable amino acid score
EAAs - “essential” amino acids,
GPC - gas permeable chromatography,
HPLC – high permeable liquid chromatography,
IMF - intramuscular fat,
JBP - Jiaying Black Pig,
LC - liquid chromatography,
LM - *Longissimus muscle*,
MUFAs - monounsaturated fatty acids,
PMQA - pig meat quality assessment,

NEAAs - "non-essential" amino acids,
PUFAs - polyunsaturated fatty acids,
SFAs - saturated fatty acids,
UFAs - unsaturated fatty acids,
WHC - water holding capacity.

1. Introduction

Meat (for human consumption) is a source of valuable proteins (including peptides and amino acids), fat (including triacylglycerols and other lipids), numerous mineral and extractive substances, vitamins, the presence of which is necessary for the nutrition and normal functioning of all mammals [1-5]. There are also L-carnitine, coenzyme Q₁₀, carnosine, taurine, creatine, glutathione, lipoic acid, conjugated linoleic acid, Omega-3 and Omega-6 polyunsaturated fatty acids (PUFAs), etc., among the known biologically active components (BACs) of meat, that can promote human health and act as the supplements in a meat diet [1-5]. The antioxidant capacity of these BACs in meat allows inhibiting oxidative processes in the human body, which can cause the progression of various diseases [6]. Some meat BACs exhibit an antihypertensive effect, preventing cardiovascular diseases, etc. [3-6].

The biological value of meat proteins largely depends on the ratio of "essential" amino acids (EAAs) in their samples (lysine, methionine, tryptophan, phenylalanine, leucine, isoleucine, threonine, valine, arginine and histidine), which cannot be synthesized by humans or animals, i.e. must be supplied by diet [3-6]. At average, an adult should receive about 1.0-1.2 g of protein per 1 kg of body weight with food during the day (depending on physical activity), and at least 30% of a person's daily protein diet should be completed by proteins containing all essential amino acids [4,5]. Tryptophan, lysine, methionine are especially deficient in this sense [3-6]. All other (so called "non-essential or replaceable") amino acids (NEAAs) are also valuable for the human body, i.e. they perform various functions and play same important roles, as well as EAAs. For example, NEAA like glutamic acid is the most important acid that supports the neurotransmission, respiration of brain cells, etc. [7]. There is a strong evidence that some of the NEAAs play an important regulatory role in gene expression, cell signaling, nutrient metabolism, and even in animal reproduction [8].

On the other hand, a novel concept of "functional" amino acids has appeared recently, pointed the AAs whose functions are not limited to protein synthesis [8,9]. This concept is proposed to highlight exactly those amino acids (both, NEAAs and EAAs) that regulate key metabolic processes in the body, contribute to improving health, safety, growth, development, reproduction function, productivity, etc. [8,9]. For example, arginine, serine, tyrosine, cysteine, and glutamic acid are referred to the "functional" amino acids with multiple physiological functions [6,9]. In this sense, the authors of this review proposed to mention such AAs as "multifunctional" instead of simply "functional", that would better reflected their numerous valuable properties.

Meat is also considered to be the main source of important unsaturated fatty acids (UFAs) in the human diet [1-5]. For example, among the three essential polyunsaturated fatty acids, arachidonic acid has the highest biological activity (2-3 times higher as those for linoleic or linolenic acids). Its absence or deficiency in the human diet delays physiological development [10]. In the human diet, nutritionists pay attention to the ratio of polyunsaturated (PUFA) to saturated (SFA) fatty acids, which should tend to a level above 0.4, but such analysis is rare for animals [5]. Modern study in human diet is paying a great attention to the content of particular PUFA, as well as the balance between ω -3 PUFA (18: 3) and ω -6 PUFA (18: 2) in the nutrition BACs [11].

Pork is a valuable food product for people, which has good nutritional and taste qualities. It is important that pork retains the valuable properties during any processing and conservation (production of sausages, smoked meats, etc.). In terms of meat popularity in Russia, pork is not inferior to beef and lamb, but a lit bit lower than poultry. Pork contains less than other types of meat, such proteins as collagen and elastin (with less EAA as other essential proteins) [12]. The quality of pork, including its chemical and technological properties, largely depends both, on the amino acid composition of muscle tissue and the ratio of various fatty acids in adipose. Improving the quality of meat and "relative" products (obtained from it) is an urgent task facing agricultural producers and

processors. That is why regulation of the composition of amino acids and lipids in pork (as well as in the meat of other productive animals) is an important problem for scientists and practitioners in the field of animal husbandry both in Russia and in the world.

The purpose of this review is to summarize the results of numerous domestic and foreign studies in the field of pork biochemistry, in particular, the fatty acid and amino acid compositions and their influence on the formation of the pork properties and quality; description of various factors, including the diet of animals; the content of these nutrients in the tissues of pigs and methods for their determination.

2. Methods for determination of amino acids and fatty acid in animal meat

The following HPLC methods are known for analyzing food products [13, 14]: ultra-HPLC, "hydrophilic" chromatography [15], two-dimensional and multidimensional HPLC methods [16 =78], chiral chromatography [17], express chromatography [18, 19], capillary HPLC [20], nano-HPLC [21], micellar HPLC [22], HPLC on monolithic columns [23], HPLC on surface porous sorbents [24], immunochromatographic methods [25], green chromatography [26], high-temperature HPLC [27]. The most common methods for the determination of amino acids and fatty acids are reversed-phase, cation-exchange HPLC and gas chromatography, respectively (Fig. 1).

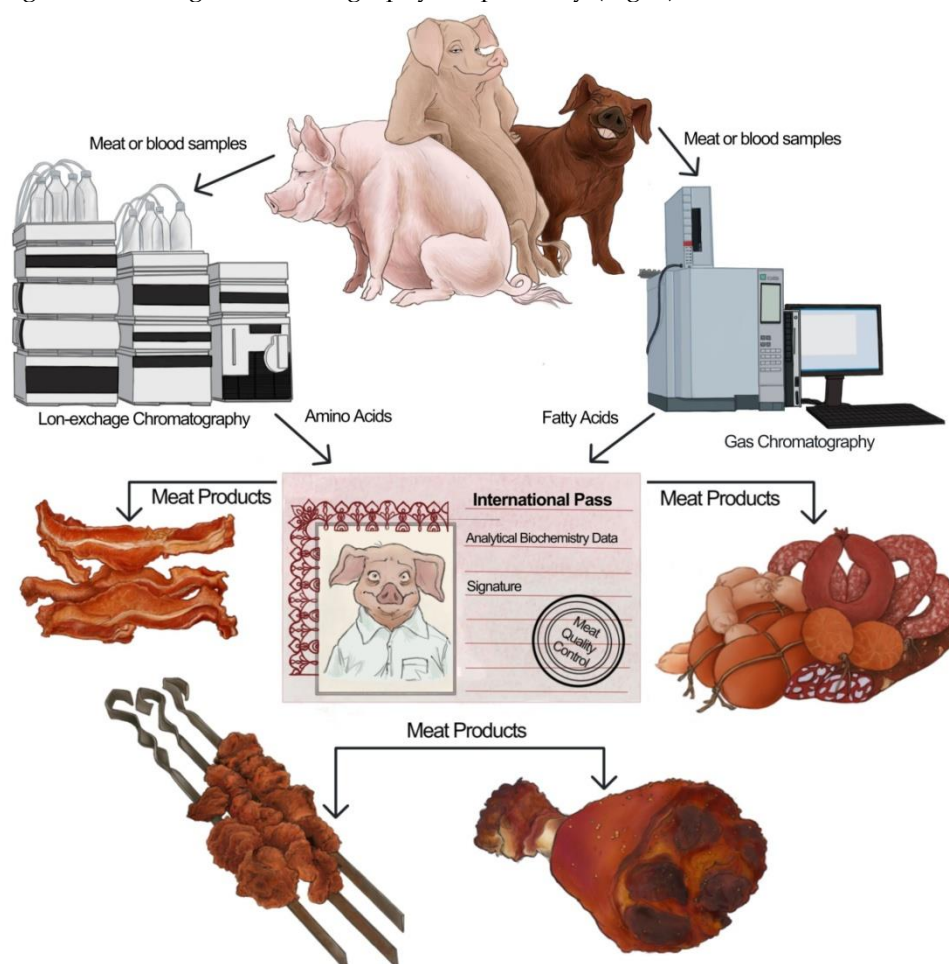


Figure 1. Schematic drawing of the chromatography methods for analyzing AA and FA content in pork blood and meat for evaluation of the food product quality.

Free amino acids are isolated from samples by extraction. Acid hydrolysis with 6 M hydrochloric acid is most often used for protein degradation [28]. The absence of chromophore groups in most amino acid molecules requires a derivatization step. Various reagents have been proposed for pre- and post-column derivatization [29, 30].

Dudkina N.N. and co-authors (2017) [31] proposed and tested a method of reversed-phase liquid chromatography (with spectrophotometric detection) for AA analysis in pig's tissues and

organs. Sample preparation included preliminary hydrolysis of proteins with 6 M/L solution of hydrochloric acid. The hydrolysates were subjected to precolumn derivatization with phenyl isothiocyanate for spectrophotometric recording on a reversed-phase column in a gradient mode. Amino acids were identified with respect to a mixture of standard samples that passed all stages of analysis [31].

An improvement of HPLC technology and its wide practical application solves the problems of separation and quantitative determination of very small amounts (10 mg/kg and below) of determined components in complex objects [32]. The authors proposed a method for determining the most important amino acids in complex matrices of biological origin, which include meat samples, by reverse phase HPLC with UV-detection using acid hydrolysis and modification of amino acids with a solution of phenyl isothiocyanate in isopropyl alcohol to obtain phenylthiohydantoin. The authors established the pH influence of the mobile phase on the separation factors of amino acids, proposed to optimize the conditions for acid hydrolysis of samples by performing a sample preparation procedure, including acid hydrolysis of samples and modification of amino acids with a solution of phenyl isothiocyanate. A hydrolysis and other technique have been described previously and the resulting AA solutions were subjected to chromatographic analysis [13-15, 31-33].

The methods for determining the amino acid and fatty acid composition of pork according to different authors are summarized in the Table 1. Analyzing the data in the table, we can conclude that the main method for determining the content of amino acids in the tissues of pigs is the method of ion exchange chromatography, fatty acids - the method of gas chromatography.

Table 1. Methods of the analysis of amino acids and fatty acid content in pork.

№	Objects, heads, genotype, etc.	Goals	Methods	Refs.
1	AA in pork meat (10 meat samples, used in the production of sausages at the enterprises of the Tambov and Lipetsk regions).	Assessment of the quality of meat obtained from various (domestic and foreign) suppliers.	Ion-exchange chromatography (analyzer "AAA - 400, Ingos").	Gubanov, D.G. 2011.
2	AA in pork meat (a few dozens of meat samples).	Assessment of the meat quality at storage.	HPLC, derivatization with orthophthalic aldehyde.	Knyazeva, A.S. 2017.
3	Content of free AA and FA in muscle tissue of rearing and fattening pigs (60 animals, Duroc x Large white x Landrace).	Assessment of the meat quality because of a change in the composition of the diet (addition of L-arginine and glutamic acid to the diet).	AA - HPLC (L-8800; Hitachi); FA - GPC (Agilent 6890N)	Guo, Q. et al., 2019.
4	Free AA and FA content in pig meat products (dry pork fillets in vacuum packing).	Assessment of the meat quality because of a change in the mixture of the probiotics.	Ion-exchange chromatography (analyzer "AAA - 400, Ingos").	Neffe-Skocińska, K. et al., 2016.
5	AA and FA content in pig meat, 47 boletus and 47 gilts, divided into groups: CChS (n = 22), BTSchS (n = 18), DBChS (n = 18), DLCCChS (n = 18), Berkshire (n = 18)/	Assessment of the meat quality of various animal genotypes.	FA - GPC (Agilent 6820; Agilent Technologies); AA - Ion-exchange chromatography (S-433D Sykam Automatic Amino Acid Analyzer).	Zhang, W. et al. 2019.
6	AA and FA content in pig meat (40	Assessment of the meat quality	FA - GPC (Agilent 6820,	Jiang, Y.

	castrated boars are divided into breed groups: aboriginal Dahe and Davout crossbreeds).	of various animal genotypes.	Agilent Technologies) using a capillary column (HP-Innowax, Agilent, length 30 cm, D=0.32 mm and film thickness 0.25 mm)	Z. et.al 2011.
7	FA content in pig meat (32 Prestice Black-Pied aboriginal pigs and 32 hybrid pigs Large White x Landrace x Duroc x Pietrain).	Assessment of the meat quality of various animal genotypes.	FA – GPC (6890N Agilent Technologies)	Nevrkla P. et.al 2017.
8	AA and FA content in pig meat (30 Landrace; 30 Landrace x Yorkshire; 30 Landrace x Yorkshire x Duroc).	Assessment of the meat quality of various animal genotypes.	AA - HPLC (LC-20 Prominence, Shimadzu), FA – GPC (Hewlett Packard).	Neupokoeva, A.S., 2019.
9	FA content in pig meat.	Assessment of the meat quality of various animal genotypes.	FA – GPC by GOST P 51483-9999 using Agilent 7890A.	Gurinovich, G.V., et al., 2020.
10	FA content in pig meat (31 Landrace breed).	Assessment of the meat quality by changes in the composition of the diet (introduction of microalgae supplements into the diet in the amount of 7% and 5%).	FA – GPC (PerkinElmer CLARUS 680) using a capillary GC (Agilent, Santa Clara).	Kalbe, C., et al., 2019.
11	FA content in pig meat (594 pigs Landrace x Yorkshire).	Assessment of the meat quality by changes in the composition of the diet.	FA – GPC (Agilent 6890N GC, Agilent Technologies).	Gjerlaug-Enger, E., et al., 2014.
12	FA content in minced meat of pigs and their products (sausages, hams, etc.).	Assessment of the meat quality.	FA – GPC (Agilent 7890A Agilent Technologies).	Pietrzak-fiecko, R. et.al. 2014.
13	FA content in pig meat (60 Landrace pigs, 12 in each group).	Assessment of the meat quality by changes in the composition of the diet (adding flaxseed oil, vitamin E and organic selenium).	FA – GPC (Agilent GC-7890 Agilent Technologies Inc.).	Wojtasik-Kalinowska, I. et.al., 2018.
14	FA content in pig meat (30 samples of meat from Polish breeds Pulawskaya and a cross between Pulawskaya x Landrace).	Assessment of the meat quality depending on the animal husbandry system.	FA – GPC (Varian 450-GC).	Karwowska, M., et.al., 2013.
15	FA content in pig meat (60 boletus of the Korean breed)	Assessment of the meat quality depending on the animal husbandry system.	FA – GPC (Varian 3,600).	Kim, D.H et.al., 2009.

Notes: GPC - gas permeable chromatography, HPLC – high permeable liquid chromatography.

3. The influence of fatty acid and amino acid composition of pig tissues on the formation of physical-chemical properties of meat

Technological properties such as adipose tissue hardness, shelf life (oxidation of lipids and pigments) and aroma depend on the content of fatty acids in the tissue [34,35]. The authors [36] have shown that major SFA, such as stearic (18:0), palmitic (16:0) and myristic (14:0) acids, as well as the other SFA in small amount, such as margaric (17:0) and lauric (12:0) acids, give pork higher density and better taste. In addition these SFA are less susceptible to oxidation as UFA. It has been suggested [37] that the total amount of fatty acids, and not only the content of individual PUFAs, has a pronounced effect on the tenderness and juiciness of pork meat. The effect of fatty acids on hardness is due to the different melting points of fatty acids. For example, the stearic acid melts at a temperature of +69.4°C, margaric acid – +60.6°C, palmitic acid – +64.0°C, lauric acid – +44.0°C, oleic acid (18:1, *cis*-9) – +14.0°C, elaidic acid (18:1, *trans*-9) – +45.0°C, linoleic acid (18:2) – -5.2°C and linolenic acid (18:3) – -11.3°C [5]. Thus, as the unsaturation degree of PUFAs increases, the melting point decreases. The isomeric structure of fatty acids is also important. For example, *trans*-isomers of FAs or “trans fatty” acids melt at a higher temperature than their predecessors (i.e. oleic acid as compared to stearic acid) [5]. Moreover, *cis*-isomers of FAs and branched chain fatty acids have a lower melting point than normal (“straight chain”) fatty acids with the same number of carbon atoms (i.e. elaidic acid as compared to oleic acid) [5].

An influence of the composition and structure of fatty acids on the meat “shelf life” (i.e. time of validity) is explained by the fact that unsaturated fatty acids tend to oxidize the more, the greater the number of C = C double bonds in a given particular FA [35-38]. This leads to the development of rancidity with increasing “shelf life”. However, color change occurs not only because of FA oxidation, but also due to a number of other reasons [35-38]. For example, the reaction of the oxidation of oxymyoglobin (red-colored) to metmyoglobin (brown-colored) is usually occurs in parallel with rancidity. So, lipid oxidation products can promote pigment oxidation and *vice versa* that have been shown in the several studies [4,5,35-38]. All these findings are of great fundamental importance and practical value (but have not been fully understood yet) due to which a preparation of this review have been intended.

The effect of fatty acids on the taste of meat is due to the formation of volatile, odorous lipid oxidation products during cooking. The unsaturated fatty acids of phospholipids also play an important role in shaping the flavor of pork [38]. Early research showed that the fatty tissue in meat was the source of the characteristic species flavor [39].

Studies carried out in the period 1970-1980 have shown that the content of fatty acids C18:0 and 18:2 determines the elasticity of adipose tissue [35]. When the composition of fatty acids in meat was altered by changes in diet, genetics, gender, or body condition, these acids were highly correlated with elasticity measured subjectively (finger test) or objectively (using a compression tester or penetrometer). The role of the 18:0 to 18:2 ratio in predicting meat hardness is described by Whittington, F. M. et al. [40]. After slaughter, various changes favoring oxidation occur in pig meat, for example, the release of iron from cells and a decrease in the activity of the enzyme glutathione peroxidase [41]. Since pork contains a relatively large amount of unsaturated lipids, attempts to further increase the concentration of PUFA will increase a risk of the formation of a large number of oxidation products, leading to unpleasant odors, aromas and discoloration. This becomes the reason for the discussion of many authors [42,43,44].

Larick D. K. et al [45] showed that pork with a high content of C18:2 acids rapidly oxidizes when heated, forming various volatile compounds, including the aldehydes pentanal and hexanal. The authors [45] did not observe any changes in the aroma and color of minced pork products prepared from meats with low, as well as with high content of C18:2 fatty acids [45]. Hartman et al. [46] studied an increase in the concentration of C18:2 from 15% to 33% of the total amount of fatty acids in pork and found no significant effect on the taste and color of pork products [46]. Since pork contains a sufficiently high concentration of PUFAs (such as C18:2 and others), the products of their

oxidation will influence the natural flavor of pork [47, 48]. The major results of the FA composition evaluation of pig muscle tissue are summarized in the Table 2.

Table 2. Content of the fatty acids in pork (according to some authors, cited in the last column).

№	Pig breed	Fatty acid content					Ref.
		Total amount or SFA+MUFA*	SFAs	MUFAs	PUFAs	Ratio SFA/UFA	
1	Duroc x Large White x Landrace (DLwL)	2031-3962* mg/100 g	767-1433 mg/100 g	840-1779 mg/100 g	418.5-743.3 mg/100 g		Guo, Q. et.al. 2019 [49]
2	Jiaxing Black Pig (JBP)	92.03 mg/g	41.63 mg/g	50.40 mg/g	7.97 mg/g	0.71	Zhang, W. et.al. 2019. [50]
3	Berkshire x JBP (BJBP),	90.67 mg/g	40.63 mg/g	50.04 mg/g	9.33 mg/g	0.68	Zhang, W. et.al. 2019 [50]
4	Duroc x Berkshire x JBP (DBJBP)	89.91 mg/g	39.52 mg/g	50.39 mg/g	10.09 mg/g	0.65	Zhang, W. et.al. 2019 [50]
5	Duroc x Landrace x JBP	91.03 mg/g	39.54 mg/g	51.49 mg/g	8.97 mg/g	0.65	Zhang, W. et.al. 2019 [50]
6	Berkshire	87.95 mg/g	40.50 mg/g	47.45 mg/g	12.05 mg/g	0.68	Zhang, W. et.al. 2019 [50]
7	Aboriginal breed Dache and hybrids Davu	106.47-108.08* mg/g 86.5-94.6** mg/g	42.91-42. 99 mg/g	43.63-51.67 mg/g	11.81-21.53 mg/g	SFA/UFA 1.48-1.52 MUFA/SFA 0.28-0.50	Jiang, Y.Z. et.al .2011[51]
8	Chinese aboriginal breed Prestice Black-Pied breed (PBP) and hybrids Large white x Landrace x Duroc x Pietrain		36.76-38. 59 %	47.86-50.27 %	12.97-13.55 %	MUFA/SFA 1.24-1.37 MUFA/PUFA 3.53-3.88 SFA/UFA 0.35	Nevrkla P. Et.al 2017 [52]
9	Landrace		451.76-50 1.20 mg/100 g	507.33-523.43 mg/100 g	312.49-370. 67 mg/100 g	omega-6/ omega-3 1.46-6.54.	Kalbe, C et.al.,2019. [53]

10	Landrace	41.6-44.9 9 %	35.13-41.42 %	12.57-19.98 %	MUFA/SFA 1.23-1.41 MUFA/PUFA -3.59-4.62	Wojtasik-Kalinowska, I. et.al., 2018. [54]
11	Pulawskaya x Hybrid Pulawskaya x Landrace	38.51-41. 38 %	53.16-58.04 %	2.67-4.42%		Karwowska, M., et.al., 2013 [55]
12	Corean aboriginal breed	34.61-37. 16 %	42.48-48.37 %	14.47-22.91 omega-3 0.47-1.84, omega-614. 0-21.7%	omega-6 / omega-3 11.92-25.33. MUFA/PUFA 1.23-1.70 MUFA/SFA 0.39-0.66	Kim, D.H et.al., 2009. [56]

Note 1: * calculated by the cited authors; ** calculated by us.

Note 2: EFAs are saturated fatty acids, MUFAs are monounsaturated fatty acids, PUFAs are polyunsaturated fatty acids, EFAs / UFAs are the ratio of unsaturated fatty acids to unsaturated ones, UUFAs / EFAs are the ratio of unsaturated fatty acids to saturated ones, MUFAs / unsaturated fatty acids are the ratio of monounsaturated acids , PUFA / EFA is the ratio of polyunsaturated acids to saturated ones.

Note 3: Animal cross-breeds: Duroc x Large White x Landrace (DLwL); Jiaying Black Pig (JBP), Berkshire x JBP (BJBP), Duroc x Berkshire x JBP (DBJBP), Duroc x Landrace x JBP (DLJBP).

There are numerous authors determined the total FA content, as well as the fractions as EFA, MUFA, PUFA and their ratios in pork [39-47]. It should be noted that the genotype of animals leaves an imprint on the fatty acid composition of the muscle tissue of pigs. Thus, according to (Guo, Q.et.al 2019 [49]), the total content of fatty acids in the pig muscle tissues of cross-breeds Duroc x Large White x Landrace is about 2031 -3962 mg / 100 g of meat (Table 2). According to another author (Jiang, Y. Z. et.al., 2011 [51]), the FA content in the muscles of Chinese aboriginal breeds was 106.47-108.08 mg / g. The ratio of EFA to UFA in the muscle tissue of pigs of different genotypes ranges from 0.65 to 0.71 units. According to Wojtasik-Kalinowska, I. et.al. [54], the ratio of MUFA / EFA in the muscle tissue of Landrace pigs was 1.23-1.41 units, while according to Jiang, Y.Z et.al. [51], this indicator in pigs of indigenous Chinese breeds was higher and amounted to 1.48-1.52 units. The ratio of omega-6 to omega-3 fatty acids is at the level of 1.46-25.33 units according to different authors [53-56] and also depends on genetic factors and conditions of animal farming.

Proteins of muscle tissue determine (to the greatest extent) its biological value, as well as the most important properties of meat in general (consistency, taste, color). The need to further study the amino acid profile of pig muscles is due to the fact that many of the amino acids are strong flavoring agents and amino acid analysis can predict the properties of finished products. The composition of muscle tissue includes proteins both: of muscle fibers and of the intercellular environment [3-5]. It is known that the muscle fiber proteins are part of the membrane (sarcolemma), nuclei, sarcoplasm, myofibrils and other structural elements of the cell [3-5]. They can be in a free state or associated with lipids, cations, glycogen, adenosine triphosphate (ATP) and other compounds [3-5]. Approximately 40% of muscle tissue proteins are soluble in water (sarcoplasmic proteins and, partially, myofibril proteins) and above 40% - in solutions of potassium chloride of medium concentration (myofibril proteins) [57].

The indicators characterizing the biological value of AA content of proteins are developed by the FAO and WHO : standards of amino acid scale (1973, 1981, etc.), which simulates the "highly likely" calculated protein, i.e. so called "ideal" protein [10, FAO amino acid scoring patterns 1981 <http://www.fao.org/3/M3013E/M3013E00.htm>]; the digestible indispensable amino acid score

(DIAAS) <http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf>. Amino acids, presented in muscles, determine the taste of meat products [3-5,57-60]. For example, alanine, serine, threonine have a slightly sweet taste, while glycine, valine and proline are sweeter. Arginine, methionine, leucine, phenylalanine have a slightly bitter taste, while isoleucine, tryptophan, histidine and lysine have a bitter taste [57]. The composition of muscles contains the most glutamic acid and glutamine, which give “meaty” taste to meat [57]. Muscle proteins of pigs of various fatness differ in structure and amino acid content, which decreases with an increase in pork fat and a decrease in the amount of protein [57].

The numerous reactions between free amino acids and carbohydrates play an important role in the formation of the taste and aroma of meat, due to the multicomponent system of substances. At the same time, the amino acids that are bound to backbone in the long AA chain of biopolymer molecule do not affect significantly the taste and aroma of the product. Free nucleotides also belong to the substances involved in the formation of taste and aroma of meat. At the same duration of sterilization, the decrease in nucleotides and increase in nucleosides (inosine and hypoxanthine) and the total content of purine bases; the more intensive at higher sterilization temperature [58]. The presence of a specific smell during cooking and frying of various types of meat is due to the formed carbonyl compounds. The taste and aroma manifested when salting a meat product is due to the particular “conversion” of carbohydrates, lipids and amino acids, as well as the presence of a complex of organic compounds, which include purines, pyrimidines, creatine, creatinine and carboxylic acids [59].

The ratio of tryptophan to hydroxyproline is used as one of the most important criteria for the quality of meat products, as well as a protein quality indicator [60]. The higher this indicator, the higher the nutritional value of meat, the more muscle tissue, muscle proteins, and essential amino acids in the meat in total [60].

Table 3. The content of amino acid acids in pork (according to cited authors)

No	Breed or cross	The total AA content	Refs.
1	Duroc x Large White x Landrace	Free AA 69.35-73.93 mg/100 g	Guo, Q.et.al 2019.- [49]
2	Berkshire, Black Pig (JBP), Berkshire x JBP (BJBP), Duroc x Berkshire x JBP (DBJBP), Duroc x Landrace x JBP (DLJBP).	The total AA content 19.06-21.15 mg/100 g	Zhang, W. et.al. 2017. [68]
3	Chinese aboriginal breed Dache and hybrids Davu Landrace	The total AA content 76.15-77.46, the amount of essential AA 30.16-30.64 mg/100 g	Jiang, Y. Z. et.al 2011 [51]
		The total AA content 21.701 %, the amount of non-essential AA 13.081 %	Neupokoeva, A.S. 2019[61]
5	Landrace x Yorkshire	The total AA content 22.419 %, the amount of non-essential AA 13.546 %	Neupokoeva, A.S. 2019[61]
6	Landrace x Yorkshire x Duroc	The total AA content 22.561 %, the amount of non-essential AA 13.577 %	Neupokoeva, A.S. 2019 [61]

Notes of the animal cross-breeds: Duroc × Large White × Landrace (DLwL); Jiaxing Black Pig (JBP), Berkshire × JBP (BJBP), Duroc × Berkshire × JBP (DBJBP), Duroc × Landrace × JBP (DLJBP).

The contents of amino acids in the muscle tissue of pigs (according to some authors) are summarized in Table 2. It should be noted that the genotype of animals is also a determining factor influencing the amino acid composition of meat obtained from animals. It is amazing that the total amino acid content in muscles of aboriginal Chinese breeds and their crosses in the study by Jiang, Y.Z. et.al. (2011) [51] is 76.15-77.46 g / 100 g of meat. In the muscles of Jiaxing black pigs and their crosses with Berkshire, Duroc and Landrace, this indicator was at the level of 19.06-21.15 g / 100 g of muscle tissue. In the study of Neupokoeva, A.S. (2019) [61] in the muscle tissue of Landrace pigs and crosses with Yorkshire and Duroc, the total amount of amino acids was at the level of 21.701-22.561% and did not depend on the animal genotype. The amount of non-essential amino acids for these pigs was at the level of 13.08-13.57% and almost the same between the studied groups.

4. The influence of the most important factors on contents of pork fatty acids and amino acids

4.1 Influence of animal genotype

The authors studied the influence of the genotype on the composition of the muscle tissue of animals [62-67]. Zhang W. and co-authors [68] studied the influence of animal genotype and hybridization of the local breed Jiaxing black pig (TsChS) with Western breeds on the slaughter, meat and taste qualities of products. The authors compared 5 breeds: TsChS, Berkshire, Berkshire × TsChS (BTsChS), Duroc × Berkshire × TsChS (DBTsChS), Duroc × Landrace × TsChS (DLCCChS). Heterosis was realized in the case of DTsChS, DBTsChS and DLCCChS groups, since their carcass weight, percentage of leanness and the area of the "muscular eye" were noticeably higher as compared to the TsChS, but lower than that of the Berkshires. The internal fat content was the highest in the case of the TsChS pig group. For the hybrids of DBCCHS, muscle tissue had the highest content of essential amino acids, as well as their total amount [68].

The purposes of the experiment by Jiang Y. Z. et al. (2011) [51] were to compare the assess carcass growth and characteristics, organ weight, meat quality and intramuscular fatty acid content and amino acid composition between the Chinese Dahe Indigenous Pig and the Davou cross. Dahe pigs contained a higher percentage of carcass fat ($P < 0.001$) compared to Davou pigs. The following fatty acids : C18: 1, C16: 0, C18: 0 and C18: 2 - prevailed in the muscle tissue of both pig breeds [51].

The study of Nevrkla P. et al. (2017) [52] is devoted to comparing the quality of meat of aboriginal pig breeds and hybrid crosses. A total amount of 64 animals are participated in the experiment, 32 pigs of the Prestice Black-Pied breed (PBP) and 32 pigs of the hybrid combination Large White × Landrace × Duroc × Pietrain. Analysis of the fatty acid profile showed that there was a higher content of C8: 0 ($p \leq 0.01$), C10: 0 ($p \leq 0.01$), C15: 0 ($p \leq 0.01$), C22: 0 ($p \leq 0.01$) 0.05), C18: 1 p-9 ($P \leq 0.01$), C18: 3 Omega-6 ($P \leq 0.001$), C20: 3 n-3 ($p \leq 0.05$), C20: 4 H- 6 ($P \leq 0.01$), C22: 4 N-6 ($P \leq 0.05$), C22: 5 n-3 ($p \leq 0.01$) and C22: 6 n-3 ($p \leq 0.01$) for hybrid animals than for PBP. The opposite trend was observed in the case of C18: 1 n-9 ($P \leq 0.01$) and C20: 5 n-3 ($p \leq 0.01$). Higher MUFA content as well as MUFA/SFA ratio was found in meat from PBP breed ($P \leq 0.01$). Higher levels C10: 0 ($P \leq 0.01$), C12: 0 ($P \leq 0.01$), C14: 0 ($P \leq 0.001$), C16: 0 ($P \leq 0.001$), C14: 1 n-5 ($P \leq 0.01$), C16: 1 n-7 ($P \leq 0.05$), C18: 1 n-7 ($p \leq 0.001$), C20: 5 n-3 ($p \leq 0.01$) and C22: 6 n-3 ($P \leq 0.05$) were found in fat of hybrid pigs, however, c24: 1N-9 ($P \leq 0.01$), C18: 2 N-6 ($P \leq 0.05$), C18: N-3 ($P \leq 0.05$), C20: 4 N-6 ($P \leq 0.01$) and C22: 5 N-3 ($p \leq 0.01$) were higher in the case of PBP pigs.

Pig meat quality assessment (PMQA) of various genotypes was carried out in the work of Neupokoeva A.S. (2019) [61], in which the author assessed the biological and productive qualities of animals of Canadian selection in the Trans-Urals region. The author compared pigs of 3 genotypes - animals of the Landrace Breed, Landrace X Yorkshire (LY), Landrace X Yorkshire X Duroc (LYD). The largest amount of essential amino acids was noted in muscle tissue samples of three-breed hybrids LYD (8.984%), which is 0.364 and 0.111% more than in purebred Landrace and two-breed hybrids LY. The amino acid index and protein-quality indicator of the muscle tissue of three-breed hybrids are higher by 0.27 and 33.55% ($P < 0.05$) than in purebred Landrace and by 0.67 and 53.33% in comparison with two-breed hybrids, respectively [61].

When assessing the quality of muscle and adipose tissue obtained from pigs of various breeds, the authors determined that the fat of three-breed young was characterized by the lowest density and resistance to oxidation, where the Landrace breeds of Canadian selection and Belarusian meat were used as the paternal breeds (with a ratio of NUFA/NFA of 1.57:1 and 1.51:1). The content of the most valuable PUFA, arachidonic acid, was practically equal for animals of all studied hybrids – 0.16–0.17%. The Duroc breed has shown itself to be the best paternal breed among the studied, in terms of the effect on the technological and nutritional properties of the fat of the offspring, providing its higher density due to the lower ratio of unsaturated and saturated fatty acids –1.34 to 1 [63].

Sheiko R.I. (2019) [69] conducted research to study the quality of pig meat obtained by crossing sows of the Belarusian Large White and the Belarusian meat pig crossbred (BKBxBM) and Belarusian Large White sows with Yorkshire and Landrace breeds. The highest protein content in muscle tissue was observed in young growth (BKBxBM) xD -20.9 and (BKBxBM) xL -21.2%, respectively. The amino acid rate for all the studied amino acids was in the range of 111.3-191.4%, which indicates the content of all high-quality complete protein combinations in the young pig. In the studied samples of adipose tissue, the content of saturated fatty acids (SFA) was 38.7-45.9%, monounsaturated fatty acids (MUFA) - 44.1-46.8%, polyunsaturated fatty acids (PUFA) - 12.0-14.5% [39].

Wood J. D et al. (2004) [70] reported that the breed affects the PMQA of pigs. Pork obtained from animals of the Yorkshire breed (more fatty carcasses) had a higher concentration of C 14:0 and C 16:0, while meat obtained from the Duroc and Large white pigs (more meat carcasses) contained more polyunsaturated fatty acids. The meat of the Duroc pig had high concentrations 20:5 ω -3 and 22:6 ω - 3 [70]. The study of the influence of the genotype on the quality of meat, in particular fatty acid and AC of pork, are also devoted to the work of other authors [71,72].

4.2. Conditions for keeping animals

Consumers are now becoming more demanding about food, choosing safe and quality food sources to maintain and improve their health. In this regard, the production of so-called "organic" products has grown in recent years, because they are in great demand as food products [73]. It is believed that the introduction of the system of "organic pork" production contributes to obtaining not only high-quality products, but also with a higher nutritional value [74,75]. According to some authors, there are differences in fatty acid composition between meat obtained as a result of "organic pork" production and from animals raised under traditional conditions [76]. The authors report on the advantage of "organic pork" in terms of a higher content of polyunsaturated fatty acids in such meat and a lower content of saturated ones [77]. Other authors report that the meat of pigs raised using "organic pork" production is significantly richer in α -linolenic acid and the total amount of polyunsaturated fatty acids and has a more favorable ratio of acids ω -3 to ω -6 [78]. The results obtained by the authors indicate that "organic" meat may be more prone to lipid oxidation [75, 77].

The work of Karwowska M. et al. (2013) [55] is devoted to a comparison of the quality of meat of animals raised in organic production and under normal conditions. The authors showed that "organic" pig meat was characterized by higher lipid stability during the entire shelf life compared to meat obtained from animals raised under normal production conditions.

Kim D.H. et al (2009) also studied the effect of feeding and housing under traditional and organic conditions on carcass performance, meat quality, and longissimus muscle of Korean pigs (n = 30). Organic content contributed to the production of pork (Pb0.05) with a higher content of polyunsaturated fatty acids (PUFA) and unsaturated fatty acids, as well as a higher content of N-3 PUFA than regular (Pb0.05) Content (%) EFA 34.61 -37.16, NNFA - 62.84-65.36, MNFA - 42.48-48.37, PUFA - 14.47-22.91, omega -3 PUFA 0.47-1.84, ω -6 PUFA 14.0-21.7, ω 6 / ω 3 -11.92-25.33, MUFA / EFA -1.23-1.70, PUFA / EFA - 0.39-0.66 [56].

Grela, E.R. and Kowalczyk, E. (2009) analyzed the nutrient content and fatty acid profile of meat from conventional and organic pigs. Meat from organically grown pigs had a higher nutrient content than that from conventional pigs [78].

The influence of the keeping system on the quality of meat was also studied in her work by Semenikhina T.V. and coauthors (2012). Their research was devoted to the study of the quality

indicators of meat obtained from pigs raised in pig farms and private farmsteads. Pork obtained from animals raised on pig farms contained a higher amount of protein in muscle tissue due to balanced feeding, which allows intensifying pork production by increasing the biological and nutritional value, in particular protein and essential amino acids [79].

4.3. Animal age

It is known that AA, taste and tenderness of meat are finally formed in pigs by 8 months, when their live weight reaches 100-112 kg. In the carcasses of older pigs, there is a significant mass of adipose tissue that is not desirable for the product quality, as well as the formation of more fat than muscles [57].

With the age of animals, as the subcutaneous fatty tissue develops and changes in its composition, it becomes more "condensed", without a clear separation of layers. The separation of layers in the adipose tissue of pigs is undesirable, since it spoils the presentation of fresh meat and products from it. Studies have shown that the connectivity of adipose tissue, as well as the toughness of meat, closely correlates with the content of water, collagen, the concentration of fatty acids C 18: 0 and C 18: 2 [41].

4.4. Changes in the composition of pig meat under various storage and processing conditions

Various authors have evaluated the AA of meat under different storage conditions. So, Knyazeva A.S. and co-authors (2017) [80] evaluated the change in the amino acid composition of meat protein during long-term storage of raw materials at low temperatures. The main effect on the change in the protein substances of meat and the amino acid composition associated with them is exerted by the freezing process. A significant result of this storage can be protein denaturation and aggregation, but the subsequent storage of meat in a frozen state has a lesser effect on these changes. It was found that the most significant changes occur during storage of frozen meat for more than 180 days of storage, which can affect its technological properties. By meat storing for more than 480 days, methionine became the limiting amino acid for pork [80].

Pietrzak-fiecko R. and co-authors (2014) [81] compared the FA content of minced meat and various meat products - in ham, sausage. Among all fatty acids contained in meat products, the largest share falls on C18: 1 n-9, C16: 0, C18: 0, C18: 2 H-6 and C16: 1. PUFA makes up 8.7, 9.2 and 9% of the total amount of fatty acids, and linolenic acid - 0.6, 0.7 and 0.3% in ham, sausages and minced meat, respectively. Minced meat had the highest ω -6 / ω -3 ratio (22.5) compared to ham and sausages (15.5 and 13.9, respectively) [81].

Gurinovich G.V. and co-authors (2020) [82] studied the composition of pork according to the carcass category. The objects of research were "uncooked" smoked hams of two groups: 1) the first group hams are made from raw materials based on cutting carcasses of pigs of the 4th category, 2) the second group hams - from raw materials based on cutting carcasses of sows of the 2nd category. For cutting experiments, 3 carcasses of each category weighing 225-240 kg (category 4) and 54-63 kg (category 2) were taken [82]. The study showed that the process of fermentation of raw smoked hams (made from raw materials) from the cutting of heavy sow carcasses develops traditionally, as evidenced by the results of determining the active acidity, weight loss and water activity. Fermented hams are characterized by higher protein content than hams made from "young" meat under similar conditions. The peculiarities of the fatty acid composition of minced ham contribute to a more intensive development of the lipid degradation process. This is evidenced by the results of determination of free fatty acids, primary and secondary oxidation products, which did not have a negative impact on the organoleptic properties of hams and safety indicators [82].

Summarizing the data discussed above, we can conclude that genotypic and phenotypic factors affect the quality of meat. Obtaining high quality livestock products, rich in essential nutrients, is currently very relevant and in demand, due to the influence of the composition of meat on the health of the population consuming products. In this regard, the development of strategies for the enrichment of livestock products with nutrients and obtaining high-quality functional meat is paid much attention in the works of numerous authors [83-91] that will be discussed below.

4.5 Regulation of pork composition using nutritional factors

Several authors [64, 65, 69] have noted that the addition of various oils to pig diets is an effective way to alter the fatty acid composition of pork. The possibility of modifying intramuscular fat in

pork can lead to an increase in the proportion of unsaturated fatty acids [64]. Some authors [65, 69] report that feeding animals with diets rich in fat usually results in higher levels of PUFA in muscle, but this affects negatively the taste of the product [65]. So, the lipid composition of the diet of animals has a decisive effect on the taste of meat obtained from them [69].

Wojtasik-Kalinowska I. et al. (2018) [54] studied the fatty acid profile of the muscle tissue of Landrace pigs after feeding a diet with various ingredients. The results showed that when the main diet was fed to animals with the addition of 3% flaxseed oil, 100 mg per kg vitamin E and 1 mg per kg organic selenium, the level of polyunsaturated fatty acids (PUFA) in meat samples was 26% higher ($P \leq 0.05$) as compared to control [54]. By adding the fat flaxseed (5%) in diets for "organic pork" production increased significantly the level of linolenic acid in muscle fat, as compared to animals fed conventional diets supplemented with 2% soybean oil [51].

Several other studies [68, 69, 71] have examined the effect of the inclusion of various oils (such as soybean, peanut, corn and sunflower oils), containing a high proportion of C18:2 fatty acids in animal diets on the acid composition and quality of pork. Studies have shown that the concentration of C18:2 acids in meat can be easily raised from about 10-15% fatty acids to over 30% with these nutritional factors [68,69,71].

Changing the diet of pigs by adding C18:3 fatty acids (from rapeseed and linseed oils) has been studied in the works of several authors [73-75] in order to reduce the ratio $\omega-6/\omega-3$. In some studies, the authors did not note any negative changes in meat quality when these products were included in animal diets [74], while in others, the meat had an unpleasant odor and taste, especially when salt was added, chopped, frozen, and reheated [75]. Campo M. M. et al. (2003) showed that C 18:3 fatty acids produce more intense odors than C18:2. [91]. Several authors [83-85] reported a positive effect of antioxidants fed together with sources of fatty acids to improve the fatty acid composition of pork, as well as the meat quality during storage.

Several other authors have studied ways to improve the quality and oxidative stability of pork using nutritional factors [92-97].

Hwang, Y. H. et al (2010) [95] fed Landrace x Yorkshire x Duroc hybrids with different doses of "betaine" 40 days before slaughter. The study of the fatty acid composition of pork showed that changes in the composition of the diet contributed to an increase in the proportion of saturated fatty acids and a decrease in unsaturated ones [95].

Zhong, J.-F. et al (2018) [96] fed Landrace x Yorkshire x Duroc hybrids for 30 days with a probiotic complex consisting of inactivated bacteria of the genus *Mycobacterium phlei*, which has immune-stimulating properties. Fatty acid analysis of the *Longissimus dorsi* showed a slight decrease in saturated and an increase in monounsaturated and polyunsaturated fatty acids. At the same time, an improvement in the quality of meat was observed with an increase in the content of intramuscular fat due to the inactivation of some genes [96].

The optimal content of fatty acids in the muscles (both, from neutral fats and phospholipids) and the presence of many fatty layers passing through the entire pulp, which forms a "marble pattern" in the meat (called "marbling" in cooking), determines all the meat quality indexes, especially - juiciness and tenderness.

Guo Q. et.al. (2019) [49] studied the effect of such "functional" amino acid as arginine on the quality of meat. In the diet of 60 growing and fattening pigs (Duroc x Large white x Landrace) during 2 months before slaughter: 1.0% of arginine and glutamic acid were additionally introduced. After the slaughter of animals, a comparative study of AA and FA profiles in muscles was carried out. It was shown that these fed supplements had an insignificant effect on the content of free amino acids, did not affect the content of 5-nucleotides and the taste of meat. The addition of arginine and glutamic acid significantly increased ($P < 0.05$) the accumulation of fat and the content of fatty acids in the muscles, promoted an increase ($P < 0.05$) in the formation of fatty acid oxidation products, improved tenderness and juiciness of meat. This study [49] showed that feed additives containing 1.0% L-arginine and glutamic acid can be used to improve the quality of pork.

The use of arginine feed additives for animals promoted an increase in intramuscular fat [79], muscle growth and reduced fat accumulation in pigs [80], improving meat quality and yield. It was

also noted that the addition of arginine to diets lowers the regulation of glycolysis in muscles after slaughter and increases the pH (from 4 to 5 units) in muscles during slaughter [80].

Kalbe C. et al (2018) [53] investigated the effect of long-term intake of microalgae supplements (7% in the diet of piglets and 5% in the diet of fattening) on the muscle microstructure and meat quality, including FAs in Landrace gilts (n=31). An increase in the concentration of ω -3 and ω -6 polyunsaturated fatty acids (PUFA) was found, which led to an increase in the accumulation of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the meat of pigs receiving this supplement [53].

The inclusion of dry apple pomace (of 10-20% of the nutritional value of the main diet) in the diet of fattening pigs, as a partial substitute for compound feed, did not have a significant effect on the amino acid composition of pork, but contributed to a significant increase in the content of valine, methionine, isoleucine, leucine, phenylalanine, serine and cystine in meat [82].

In the works of other authors, attention is paid to the amino acid composition of animal muscle tissue [98-104].

Thus, the lifetime formation of high quality pork is possible by using various nutritional factors in the diets of animals, which, according to many authors, can serve to change the composition, taste and technological properties of meat, especially the FA content and their ratio. The comparative changes in the amino acid composition of pork by changing the diets of cross-breed animals are almost not shown in the available literature sources.

5. Conclusions

Meat is a source of proteins (including peptides and amino acids), fat (including triacylglycerols and other lipids), numerous mineral and extractive substances, vitamins, the presence of which is necessary for the normal functioning of the body. The quality of pork, including its chemical and technological properties, largely depends on the amino acid composition of muscle tissue, as well as content and the particular ratios of various fatty acids in pork fat.

It is important to highlight that the total amount of fatty acids has the greatest effect on the tenderness and juiciness of pork meat, and not only the content of individual PUFAs. The effect of fatty acids on hardness is due to the different melting points of fatty acids. The effect of fatty acids on the taste and color of meat is due to the formation of lipid oxidation products during cooking and technological procedures.

Vital enrichment of the nutritional composition of pig meat is currently very important, since it is the basis of technologies for providing humans with healthy, safe and high-quality food of animal origin and contributes to the preservation and improvement of the human environment. By changing the nutritional factors, it is possible to regulate the fatty acid composition of the adipose and muscle tissue of pigs.

The main methods for determining the fatty acid and amino acid contents are the following: gas chromatography and ion-exchange chromatography, respectively.

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