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Effects of breed and slaughter age on the amino acid profile of beef from bulls of combined-purpose cattle breeds

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ABSTRACT

The article examines the qualitative composition and quantitative content of amino acids in the *Longissimus dorsi* muscle of bulls from three domestic dual-purpose breeds. Beef obtained from 15-month-old Simmental bulls exhibited the highest protein content (mass fraction of 22.17%). The analysed *Longissimus dorsi* muscle samples contained nine determined non-essential amino acids and seven essential amino acids. Cysteine was not determined because a standard solution was unavailable; tryptophan was not determined because of limitations in the method used. Among essential amino acids, lysine, arginine, threonine, and leucine accounted for the largest shares; non-essential amino acids were represented primarily by alanine, glutamic acid, and asparagine. The total content of the determined amino acids showed age-related fluctuations, increasing from 61.24-63.78% at 3 months to 73.52-84.71% at 9 months, decreasing to 60.34-72.23% at 15 months, and increasing again to 72.48-76.09% at 21 months, specifically, the proportion of essential amino acids increased from 27.80-29.73% (at 3 months) to 36.05-37.55% (at 21 months), while that of non-essential amino acids rose from 33.44-34.29% (at 3 months) to 36.43-38.54% (at 21 months).

Keywords: amino acid, bull, age, breed, beef

INTRODUCTION

In the current state of animal husbandry, improving the quality and nutritional value of products is of crucial importance **[1]**, **[2]**. Beef, with its distinctive taste, high nutrient content, favourable price-value ratio, and popularity, has become a go-to choice in the meat market. However, it meets only 22% of demand. Beef contains a balanced composition of proteins, fats, minerals, vitamins, essential amino acids, and fatty acids **[3]**, **[4]**, **[5]**. The worldwide yearly gross production is approximately 60 million tons. The largest beef producers globally are the United States of America, Brazil, China, and Argentina **[6]**. However, an extremely important aspect in ensuring the competitiveness of this type of meat raw material in both domestic and foreign markets is to improve its quality, which depends on some different factors that have a significant impact both on the animal during rearing and on the carcass after slaughter **[7]**, **[8]**. Among them are breed characteristics **[9]**, **[10]**, age **[11]**, sex, physiological state of the animal, and feeding and housing conditions **[12]**, **[13]**, **[14]**. At the same time, its biological value as a major source of high-quality, complete protein in the human diet is primarily determined by the balance of its amino acid composition, which plays a key role in supporting vital bodily functions **[15]**. Amino acids are divided into essential and nonessential. Nonessential amino acids are those that the human body can synthesise from other amino acids. Essential amino acids cannot be produced in the body and must be supplied by food or partially synthesised **[16]**, **[17]**. In this context, it is important to understand their qualitative and quantitative ratio. Proteins that contain all essential amino acids are considered complete; those lacking any of them, or containing them in small amounts, are considered deficient. Beef, a versatile meat suitable for human consumption at any age **[18]**, differs from meat from other farm animal species in its amino acid profile, mainly because it contains higher levels of balanced essential amino acids **[19]**, **[20]**. An imbalance in protein amino acid composition is usually characterised by disorders in animal protein synthesis. Excessive

amounts contribute to the formation of highly toxic metabolic products that are not used for amino acid synthesis, which can negatively affect the quality of muscle tissue **[21]**. The amino acid content in cattle muscle tissue is relatively stable. Instead, some scientific literature emphasises certain differences in this indicator depending on the breed **[22]**, **[23]**, **[10]**, sex **[24]**, **[25]**, **[26]**, age **[27]**, **[28]**, **[29]**, **[30]**, **[31]**, **[32]**, and feeding conditions **[33]**, **[22]**, **[10]**. In addition, the latter technological link, as stated by Fujimura and Kadowaki **[34]**, is one of the key abiotic factors that ensures not only the intensity of production but also the quality of the final product - meat. On the other hand, there are still controversial issues related to understanding the parameters of beef amino acid composition formation under the influence of some of the above factors, in particular breed and age, the knowledge of which will allow us to pay special attention to the physiological importance of substitutable and essential amino acids in ensuring the normal functioning of the human body. First and foremost, this applies to combined dairy breeds, which play an exceptional role in achieving high milk productivity and differ from dairy breeds in their better meat qualities. In domestic dairy farming, particularly when raising cattle for meat under typical feed and technological conditions, relevant studies are limited and therefore require more substantial scientific substantiation.

Scientific Hypothesis

The amino acid profile of the *Longissimus dorsi* muscle differs among dual-purpose cattle breeds and varies with slaughter age.

Objectives

The study aimed to determine patterns of variation in the amino acid composition of the Longissimus dorsi muscle of bulls by breed and age.

MATERIAL AND METHODS

Samples

Samples description: In accordance with the experimental protocols for control slaughter, three animals from each breed group-with live weights corresponding to the group average-were selected for each age period (3, 9, 15, and 21 months). Slaughter was carried out in the production conditions of a meat processing plant. After bleeding, skinning, and evisceration, the veterinarian first inspected the carcasses and then divided them into right and left halves. The cut carcasses were cooled in a refrigerator. After cooling the carcasses in a refrigerator at a temperature of +4 °C, samples of the *Longissimus dorsi* muscle were taken.

Sample collection: *Longissimus* dorsi muscle samples were obtained from bulls at 3, 9, 15, and 21 months of age. To determine changes in the muscle's amino acid profile by breed and age, 400 g samples were collected from each carcass at specific age intervals and at the 9-12 rib levels. The samples were then freed from connective tissue. The biomaterial was placed in hermetic "Ziploc" bags, labelled, and transported in a cooling bag with a refrigerant to the accredited laboratory of the Testing Centre of the Livestock Farming Institute of the NAAS in Kharkiv for further analysis.

Sample preparation: In the laboratory, the samples were unpacked, freed from connective tissue, and homogenised, and 10 g of each sample was taken for analysis.

Number of samples analysed: A total of 36 animal-level muscle samples were analysed (3 breeds × 4 ages × 3 animals).

Chemicals

All chemicals were purchased from well-known brands on the market and met the highest analytical standards: sodium phosphate buffer (grade A, chemically pure, LLC "LAZOR", Ukraine); alcoholic ninhydrin solution (grade A, chemically pure, Private Enterprise "Sistema Optimum", Ukraine); Nessler reagent (TU 6-09-2089-77, Joint-Stock Company "Kyiv Plant of Reagents, Indicators and Analytical Preparations "RIAL", Ukraine), sulfuric acid H_2SO_4 (grade A, chemically pure, Limited Liability Company "Khimlaborreaktiv", Ukraine), ammonium sulfate $(NH_4)_2SO_4$ (grade A, chemically pure, Private Joint-Stock Company "SUMYKHIMPROM", Ukraine).

Animals, Plants and Biological Materials

The studied biological material was collected from bulls of the Simmental (S), Lebedyn (L), and Ukrainian gray (UG) dairy breeds of combined productivity. According to the experimental protocol, 75 bulls were selected at the farm of the Livestock Farming Institute of NAAS (Kharkiv region) and divided into three groups. From the 25 bulls initially assigned to each breed group, three animals were selected for slaughter at each of the four sampling ages, resulting in 12 slaughtered animals per breed and 36 animals in total. Until the age of four months, they were kept in loose-housing group pens within a single facility, and subsequently tethered until the end of the intensive rearing period.

During the growing period (from 30 days to 21 months of age), the experimental animals were fed diets of

the same nutritional value, following detailed feeding standards, which provided an average daily gain of 900-1000 g. Feeding during the growing period was high and designed to identify opportunities to increase meat productivity and achieve a live weight of 550-650 kg at 18-21 months. The total nutritional value of the feed used by the bulls during the growing period was 48.0, 46.9, and 44.9 c of energy feed units. One energy feed unit accounted for 106, 105, and 102 g of digestible protein, respectively. The ratio of the main components of the diets (on average by groups) was characterised by the following indicators: energy-protein ratio - 13.62 g; calcium to phosphorus ratio 1.84:1; fibre content in dry matter - 22.3%. The energy value of the diet was 289 MJ per day.

Instruments

The qualitative and quantitative amino acid composition of *Longissimus dorsi* muscle samples from bulls was determined using an AAA T-339 automatic amino acid analyzer (Microtechna, Prague, Czech Republic) operated in the hydrolysate mode. Muscle tissue samples were analyzed in accordance with the operating instructions for the instrument.

Laboratory Methods

Muscle tissue samples were subjected to acid hydrolysis with 6 mol/L HCl, prepared by diluting concentrated hydrochloric acid with distilled water, at 105 °C for 24 h. After hydrolysis, hydrochloric acid was removed, and the dry residue was dissolved in a buffer solution and used for chromatographic analysis. A 100 µL aliquot of the hydrolysate was injected into the analytical column of the analyser. Amino acids were separated by ion-exchange chromatography on a column packed with OSTION LG ANB cation-exchange resin using sequential elution with citrate buffer solutions. Amino acids were eluted from the chromatographic column in the following order: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine. Under acidic hydrolysis conditions, asparagine and glutamine acid are deamidated to aspartic acid and glutamic acid, respectively; therefore, they were not determined separately. After elution from the chromatographic column, the amino acids underwent a post-column reaction with an alcoholic solution of ninhydrin (pH 5.8). The mixture of eluate and ninhydrin reagent was passed through a reactor maintained at 95–98 °C and then into a flow cell. The intensity of the resulting color was measured photometrically at a wavelength of 520 nm against sodium phosphate buffer solution, after which a chromatogram was generated. A standard mixture containing 0.25 µmol/mL of each amino acid was prepared using amino acid standards (Sigma, USA). From this mixture, a working calibration solution for the analysis of the hydrolysate was prepared, containing 0.2 µmol/mL of each amino acid analysed; its pH was adjusted to 2.2 using citrate buffer solution. Amino acids in the samples were identified by comparing the chromatographic peaks obtained for the samples with those of amino acid standards. Quantification was performed using a working calibration solution, and the content of individual amino acids in the samples was calculated by comparing the areas of their chromatographic peaks with the areas of the corresponding amino acid standard peaks. The sensitivity limit of the method was 10^{-4}% by mass, and the relative standard deviation was 0.04 (4%). Repeatability was estimated based on the difference between the results of two parallel determinations of the same sample. The maximum difference was 5.2% for glycine, alanine, lysine, proline, glutamic acid, isoleucine and histidine; 7.6% for threonine, phenylalanine, methionine, aspartic acid and leucine; 9.3% for arginine and valine; and 9.8% for serine. Cysteine was not analysed because a suitable standard was not available, and tryptophan was not determined because the analytical procedure used did not allow its determination. The total content of essential and non-essential amino acids was calculated as the sum of the determined amino acids assigned to the appropriate groups. The amino acid content was determined according to a method adapted from DSTU ISO 13903:2009 for muscle tissue analysis **[35]**, while the nitrogen mass fraction was determined according to DSTU ISO 937:2005 **[36]**. Total nitrogen was determined after wet ashing of the samples in sulfuric acid followed by analysis using Nessler's reagent. A standard ammonium sulfate solution was used to construct a calibration curve. The protein mass fraction was calculated from the total nitrogen mass fraction using a conversion factor of 6.25 and expressed as a percentage of the total mass of the *Latissimus dorsi* muscle sample. The determined amino acid content was expressed as a percentage of the protein mass fraction in the *Longissimus dorsi* muscle samples.

Description of the Experiment

Study flow: Twenty-five purebred bulls of each breed were reared under the same feeding conditions. Meat productivity was evaluated at four ages. Three representative bulls per breed and age were selected for slaughter; *Longissimus dorsi* muscle samples were collected, prepared, analysed chromatographically, and statistically compared by breed and age.

Quality Assurance

Number of repeated analyses: Each sample was analysed once; no repeat laboratory measurements were performed on the same sample.

Number of experiment replications: For each "breed × age" group of bulls, three animals were studied (n = 3).

Reference materials: The results are presented as the arithmetic mean ($\bar{x}$) ± standard deviation (SD). The SD reflects variability among the three biological replicates (i.e., among animals within the respective "breed × age" group), rather than the variability among repeated laboratory measurements.

Calibration: For quantitative amino acid determination, we used a working calibration solution; for total nitrogen, we used a standard ammonium sulfate solution. Cysteine was not determined due to the lack of a standard solution; tryptophan was not determined due to limitations of the method used.

Laboratory accreditation: The Laboratory for the Assessment of the Quality of Feed and Products of Animal Origin, which is part of the Testing Center of the Livestock Farming Institute of the National Academy of Agrarian Sciences of Ukraine, is certified for technical competence by the National Accreditation Agency of Ukraine, in accordance with the requirements of DSTU ISO/IEC 17025:2006 (accreditation certificate No. 2T621) and DSTU EN ISO/IEC 17025:2019 (accreditation certificate No. 20621) as a basic organization of the metrological service of the Ministry of Agrarian Policy and Food of Ukraine.

Data Access

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Statistical Analysis

The amino acid index was calculated as the ratio of the sum of essential amino acids to the sum of non-essential amino acids. The data were analysed using Microsoft Excel 2010 (Microsoft Corporation, USA) and STATISTICA 10.0 (StatSoft, USA). For each breed and age group, three independent samples from three animals (n = 3) were analysed. Results are presented as $\bar{x}$ ± SD, where SD describes variability among animals within each group. The normality of the data distribution was tested using the Shapiro-Wilk test. Inter-breed differences for each studied parameter were assessed separately within a specific age period using one-way analysis of variance (ANOVA), and then multiple pairwise comparisons were performed using the Tukey test. Different superscript letters within a single age period indicate statistically significant differences at $p < 0.05$.

Reporting and transparency statement

This was a comparative animal study conducted under common production and feeding conditions. Bulls were selected for slaughter based on the live weight corresponding to the group mean. Random allocation and blinding were not reported. The sample size was determined by the study design (three animals per breed at each age). No data exclusions were reported.

RESULTS AND DISCUSSION

A scientifically based approach to obtaining high-quality meat is the solution to the current challenges in cattle breeding **[37]**, **[38]**. Priority should be given to studying the amino acid composition of proteins and assessing the biological significance of beef as a food product in meeting human needs for essential and non-essential amino acids **[39]**.

Laboratory testing of *Longissimus dorsi* muscle samples from bulls of various breeds revealed a general decrease in protein mass fraction from 19.21-21.50% at 3 months to 18.00-19.12% at 9 months (Table 1).

Table 1 Formation of mass fraction of protein in the *Longissimus dorsi* muscle of bulls of different breeds, %.

Age, months	Breed		
	L (% ± SD)	S (% ± SD)	UG (% ± SD)
3	21.50 ± 0.23^{a}	19.21 ± 0.42^{b}	21.46 ± 0.10^{a}
9	19.12 ± 0.30^{a}	19.71 ± 0.22^{a}	18.00 ± 0.18^{b}
15	21.49 ± 0.12^{b}	22.17 ± 0.17^{a}	21.18 ± 0.18^{b}
21	21.20 ± 0.22^{a}	20.17 ± 0.19^{b}	20.74 ± 0.24^{a}

Notes: Simmental (S), Lebedyn (L) and Ukrainian gray (UG); $^{a, b}$ – different superscript letters within the same age group indicate statistically significant differences between breeds.

At 15 months of age, the average mass fraction of protein by breed was 21.61%, 2.7% higher than at 9 months of age. In the following postnatal age periods, this indicator approached the values obtained in the previous period, reaching 21.18-22.17% at 15 months and 20.17-21.20% at 21 months of age. Beef from 15-

month-old Simmental bulls was markedly distinguished by the highest protein content (22.17%). At the same time, in terms of the mass fraction of protein at 3 months of age, beef samples obtained from Lebedyn bulls significantly differed from samples obtained from Simmental bulls (p <0.01), and Ukrainian Gray bulls from Simmental bulls (p <0.05). At 9 months of age, statistically significant differences were observed between the Lebedyn and Ukrainian Gray breeds (p <0.01) and between the Simmental and Ukrainian Gray breeds (p <0.001); at 15 months - between the Simmental and Lebedyn breeds (p <0.01) and between the Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months – between the Lebedyn and Simmental breeds (p <0.01) and between the Simmental and Ukrainian Gray breeds (p <0.05).

Notably, the qualitative amino acid composition of all studied beef samples is identical, while the quantitative content differs (Table 2).

Table 2 Dynamics of the formation of non-essential amino acids in the composition of carcass meat proteins of bulls of combined breeds in ontogeny, % of protein.

Name of the amino acid	Age, months	Breed		
		L (% of protein ± SD)	S (% of protein ± SD)	UG (% of protein ± SD)
Asparagine acid	3	6.28 ± 0.15^{a}	5.83 ± 0.13^{a}	6.61 ± 0.30^{ab}
	9	6.61 ± 0.75^{b}	6.58 ± 0.30^{b}	9.79 ± 0.96^{a}
	15	7.53 ± 0.03^{a}	7.86 ± 0.06^{b}	6.55 ± 0.06^{c}
	21	7.44 ± 0.05^{b}	7.97± 0.05^{a}	6.63 ± 0.08^{c}
Serine	3	3.29 ± 0.09^{a}	2.79 ± 0.01^{b}	2.99 ± 0.07^{c}
	9	3.71 ± 0.47^{a}	3.18 ± 0.41^{a}	3.77 ± 0.27^{a}
	15	2.96 ± 0.01^{a}	2.73 ± 0.02^{b}	2.38 ± 0.01^{c}
	21	3.89 ± 0.10^{a}	3.72 ± 0.03^{b}	3.25 ± 0.02^{c}
Glutamine acid	3	11.18 ± 0.01^{a}	10.00 ± 0.43^{b}	11.22 ± 0.50^{a}
	9	14.03 ± 1.34^{a}	12.17 ± 1.74^{a}	12.46 ± 1.02^{a}
	15	11.16 ± 0.03^{a}	10.27 ± 0.08^{b}	9.40 ± 0.05^{c}
	21	12.46 ± 0.07^{a}	12.35 ± 0.07^{a}	10.57 ± 0.08^{b}
Proline	3	2.52 ± 0.08^{b}	4.07 ± 0.04^{a}	2.96 ± 0.15^{c}
	9	4.12 ± 0.90^{a}	3.40 ± 0.67^{a}	3.25 ± 0.04^{a}
	15	3.21 ± 0.01^{a}	2.93 ± 0.02^{b}	2.49 ± 0.02^{c}
	21	2.23 ± 0.05^{a}	2.24 ± 0.02^{a}	2.32 ± 0.03^{a}
Glycine	3	3.91 ± 0.10^{a}	3.90 ± 0.24^{a}	3.60 ± 0.22^{a}
	9	3.91 ± 0.07^{a}	3.64 ± 0.11^{a}	4.35 ± 0.47^{a}
	15	3.58 ± 0.03^{b}	4.00 ± 0.07^{a}	2.75 ± 0.01^{c}
	21	3.49 ± 0.07^{a}	4.29 ± 0.05^{b}	4.82 ± 0.02^{a}
Alanine	3	4.33 ± 0.01^{a}	4.53 ± 0.34^{a}	4.41 ± 0.30^{a}
	9	5.20 ± 0.37^{a}	4.96 ± 0.39^{a}	5.29 ± 0.31^{a}
	15	4.17 ± 0.03^{b}	4.40 ± 0.02^{a}	4.27 ± 0.03^{c}
	21	4.40 ± 0.03^{c}	5.63 ± 0.02^{b}	5.76 ± 0.04^{a}
Tyrosine	3	2.53 ± 0.01^{a}	2.31 ± 0.03^{a}	2.50 ± 0.27^{a}
	9	3.43 ± 0.38^{a}	2.91 ± 0.31^{a}	3.84 ± 0.29^{a}
	15	3.07 ± 0.01^{a}	2.99 ± 0.02^{b}	2.39 ± 0.01^{c}
	21	2.52 ± 0.05^{b}	2.34 ± 0.03^{c}	3.12 ± 0.06^{a}

Notes: Simmental (S), Lebedyn (L), and Ukrainian gray (UG); $^{a, b, c}$ – different superscript letters within the same age group indicate statistically significant differences between breeds.

In particular, the content of asparagine acid in beef samples during the growing period of bulls of all breeds is subject to minor quantitative changes and ranges from 5.83 to 6.61% at 3 months, from 6.58 to 9.79% at 9 months, from 6.55 to 7.86% at 15 months, and from 6.63 to 7.97% at 21 months. Moreover, in terms of breed differences, statistically significant differences in the corresponding indicator at 9 months of age were found between the Ukrainian Gray and Simmental breeds (p <0.01) and between the Ukrainian Gray and Lebedyn breeds (p <0.01); at 15 months – between the Simmental and Lebedyn breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); at 21 months – between the Simmental and Lebedyn breeds (p <0.001), Simmental and Ukrainian Gray breeds (p <0.001), and

Lebedyn and Ukrainian Gray breeds (p <0.001).

The accumulation of the amino acid serine in beef samples, which is linked to protein and fat metabolism in the body, was somewhat different. In particular, during the period of growing bulls, the rate of increase in its concentration almost did not change from 2.79-3.29% at 3 months to 3.25-3.89% at 21 months, although the statistical significance of the interbreed difference was quite high: at 3 months of age between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.01), and Simmental and Ukrainian Gray breeds (p <0.05); at 15 months – between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months - between the Lebedyn and Simmental breeds (p <0.05), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001).

Glutamine acid made the largest contribution to the total content of the identified non-essential amino acids in beef of the experimental breeds, with a slight tendency to increase in age dynamics: from 10.00-11.22% at 3 months of age to 12.17-14.03% at 9 months, decreased to 9.40–11.16% at 15 months, and ranged from 10.57 to 12.46% at 21 months of age. The highest glutamine acid content was recorded in Lebedyn beef samples (14.03% at 9 months of age). Statistically significant interbreed differences in glutamine acid content were found at 3 months of age between the Lebedyn and Simmental breeds (p <0.05) and between the Simmental and Ukrainian Gray breeds (p <0.05); at 15 months – between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months – between the Lebedyn and Ukrainian Gray breeds (p <0.001) and between the Simmental and Ukrainian Gray breeds (p<0.001).

From 3 to 9 months of age, an accelerated breed-specific increase in proline concentration from 2.52% and 4.07% to 3.25% and 4.12% was observed, except for the Simmental breed, and from 9 to 21 months of age, a stable slowdown to 2.23-2.32% was observed. Among the experimental breeds, the maximum percentage of proline in beef samples at the age of 3 months was in the Simmental breed (4.07%); at 9 months, in the Lebedyn (4.12%) and Simmental (3.40%) breeds; at 15 months, in the Lebedyn breed (3.21%). Statistical processing of the results showed significant interbreed differences in proline content at 3 months of age between the Simmental and Lebedyn breeds (p <0.001), Simmental and Ukrainian Gray breeds (p <0.001), and Lebedyn and Ukrainian Gray breeds (p <0.01); at 15 months – between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001).

The quantitative content of the amino acid glycine, which neutralises benzoic acid in the body, is relatively stable during bull growth: 3.60-3.91% at 3 months and 4.29-4.82% at 21 months. At the same time, the highest glycine concentration was found in muscle proteins of Ukrainian Grey bulls at 9 months of age (4.35%), Simmental bulls at 15 months (4.00%), and Ukrainian Grey bulls at 21 months (4.82%). Meanwhile, the concentration of amino acids in beef samples from the Lebedyn breed hardly changed with age, amounting to 3.91% at 3 months and 3.49% at 21 months. The peculiarity of the amino acid composition of muscle tissue protein of the experimental groups is the revealed interbreed significant difference in glycine content at 15 months between Simmental and Lebedyn breeds (p <0.01), Simmental and Gray Ukrainian breeds (p <0.001), Lebedyn and Gray Ukrainian breeds (p <0.001), at 21 months – between Gray Ukrainian and Simmental breeds (p <0.001), Simmental and Lebedyn breeds (p <0.001), and Ukrainian Gray and Lebedyn breeds (p <0.001).

Amino acid tyrosine is a precursor in the biosynthesis of adrenaline and insulin hormones. The amplitude of its content in beef samples across all experimental groups shows a similar trend relative to the initial level and with the extension of the bulls' growth period: 2.31-2.53% at 3 months and 2.52-3.12% at 21 months. However, the course of this process differed by breed. In particular, the highest tyrosine concentration was recorded in Lebedyn beef at 3 months (2.53%), Ukrainian Grey at 9 months (3.84%), Lebedyn at 15 months (3.07%), and Ukrainian Grey at 21 months (3.12%). It should be noted that at 15 months of age, the difference in the content of this amino acid between the Lebedyn and Simmental breeds (p <0.01), Lebedyn and Ukrainian Gray breeds became statistically significant (p <0.001), and Ukrainian Gray breeds (p <0.001); and at 21 months of age - between Ukrainian Gray and Lebedyn breeds (p <0.01), Ukrainian Grey and Simmental breeds (p <0.001), and Lebedyn and Simmental breeds (p <0.01).

Considering alanine, which supports protein and carbohydrate metabolism, improvements in beef quality do not occur simultaneously and depend on breed. The alanine concentration in beef samples increased from 4.33-4.53% at 3 months of age to 4.40-5.76% at 21 months of age. The highest concentrations were found in Ukrainian Gray beef samples at 9 and 21 months of age (5.29% and 5.76%, respectively). Statistically significant differences were found between the Ukrainian Gray and Simmental breeds (p <0.01), the Lebedyn and Simmental breeds (p <0.001), and the Lebedyn and Ukrainian Gray breeds (p <0.01) at 15 months of age, as well as between the Ukrainian Gray and Lebedyn breeds (p <0.001), the Ukrainian Gray and Simmental breeds (p <0.01), and the Simmental and Lebedyn breeds (p <0.001) at 21 months of age.

The differences in amino acid content observed between breeds in this study are generally consistent with the findings of **[22]**, **[23]**, **[10]**, who also reported variations in beef amino acid composition among cattle breeds. Similar age-related variations in individual amino acid content have been described in **[27]**, **[28]**, **[29]**, **[30]**, **[31]**, **[32]**.

The *Longissimus dorsi* muscle of bulls was most enriched in serine, proline, alanine, tyrosine, lysine, histidine, arginine, methionine, and leucine. The differences among them within the studied breeds were statistically significant, probably due to the formation of beef protein quality specific to each breed.

Table 3 Formation of the determined essential and conditionally essential amino acids in the composition of meat proteins of bulls of combined breeds in ontogeny, % of protein.

N+ ame of the amino acid	Age, months	Breed		
		L (% of protein ± SD)	S (% of protein ± SD)	UG (% of protein ± SD)
Lysine	**3**	4.99 ± 0.11^{b}	5.46 ± 0.12^{a}	5.02 ± 0.40^{ab}
	9	7.91 ± 0.91^{a}	6.83 ± 0.60^{a}	7.39 ± 0.44^{a}
	15	6.50 ± 0.02^{a}	5.75 ± 0.02^{b}	4.99 ± 0.02^{c}
	21	6.40 ± 0.09^{a}	6.04 ± 0.11^{b}	6.05 ± 0.04^{b}
Histidine	**3**	2.65 ± 0.18^{a}	2.00 ± 0.08^{b}	2.95 ± 0.21^{a}
	9	4.28 ± 0.47^{a}	3.43 ± 0.32^{a}	3.45 ± 0.12^{a}
	15	3.13 ± 0.01^{a}	3.28 ± 0.03^{b}	2.52 ± 0.02^{c}
	21	3.10 ± 0.16^{c}	3.40 ± 0.06^{b}	3.68 ± 0.05^{a}
Arginine	**3**	3.36 ± 0.16^{a}	3.60 ± 0.20^{a}	3.68 ± 0.25^{a}
	9	6.64 ± 0.84^{a}	5.30 ± 0.32^{a}	6.07 ± 0.53^{a}
	15	5.77 ± 0.02^{a}	5.49 ± 0.04^{b}	4.45 ± 0.01^{c}
	21	4.67 ± 0.12^{a}	4.90 ± 0.05^{a}	4.78 ± 0.06^{a}
Threonine	**3**	3.61 ± 0.10^{a}	2.86 ± 0.07^{b}	3.19 ± 0.27^{ab}
	9	5.02 ± 0.50^{a}	4.53 ± 0.73^{a}	4.58 ± 0.25^{a}
	15	4.06 ± 0.01^{a}	3.65 ± 0.04^{b}	2.88 ± 0.01^{c}
	21	4.08 ± 0.02^{c}	4.96 ± 0.04^{a}	4.27 ± 0.06^{b}
Valine	**3**	3.07 ± 0.03^{a}	2.99 ± 0.03^{a}	3.20 ± 0.21^{a}
	9	3.89 ± 0.45^{a}	3.50 ± 0.30^{a}	3.61 ± 0.29^{a}
	15	3.67 ± 0.01^{a}	3.49 ± 0.03^{b}	2.85 ± 0.02^{c}
	21	3.42 ± 0.04^{b}	3.77 ± 0.04^{a}	3.24 ± 0.03^{c}
Methionine	**3**	1.17 ± 0.19^{a}	1.02 ± 0.07^{a}	1.15 ± 0.21^{a}
	9	1.99 ± 0.17^{a}	1.28 ± 0.24^{b}	1.89 ± 0.14^{a}
	15	1.69 ± 0.01^{a}	1.68 ± 0.01^{a}	1.26 ± 0.01^{b}
	21	1.82 ± 0.05^{a}	1.58 ± 0.07^{b}	1.46 ± 0.02^{b}
Isoleucine	**3**	2.86 ± 0.08^{a}	2.46 ± 0.23^{ab}	2.44 ± 0.04^{b}
	9	3.18 ± 0.09^{b}	3.22 ± 0.20^{b}	4.13 ± 0.08^{a}
	15	3.12 ± 0.01^{a}	3.48 ± 0.04^{b}	2.80 ± 0.01^{c}
	21	3.06 ± 0.07^{c}	3.46 ± 0.03^{b}	4.19 ± 0.02^{a}
Leucine	**3**	5.17 ± 0.06^{a}	4.65 ± 0.22^{a}	4.79 ± 0.43^{a}
	9	6.60 ± 0.83^{ab}	5.18 ± 0.30^{b}	7.09 ± 0.45^{a}
	15	5.07 ± 0.01^{c}	5.26 ± 0.07^{b}	5.54 ± 0.01^{a}
	21	5.82 ± 0.08^{b}	5.83 ± 0.05^{b}	6.45 ± 0.07^{a}
Phenylalanine	**3**	2.86 ± 0.01^{a}	2.77 ± 0.03^{a}	2.90 ± 0.14^{a}
	9	3.20 ± 0.29^{a}	3.42 ± 0.31^{a}	3.73 ± 0.31^{a}
	15	3.54 ± 0.01^{a}	3.46 ± 0.03^{b}	2.82 ± 0.02^{c}
	21	3.68 ± 0.02^{a}	3.61 ± 0.01^{b}	3.31 ± 0.03^{c}

Notes: Simmental (S), Lebedyn (L), and Ukrainian gray (UG); $^{a, b, c}$ – different superscript letters within the same age group indicate statistically significant differences between breeds.

The content of lysine in the *Longissimus dorsi* muscle of bulls of all breeds, which is part of the sperm proteins necessary for the synthesis of haemoglobin and skeleton formation, naturally increases from 4.99-5.46% at 3 months to 6.83-7.91% at 9 months of age, decreasing to 4.99-6.50% at 15 months, and ranging from 6.04 to 6.40% at 21 months of age (Table 3).

Instead, its highest concentration (7.91%) was recorded in samples of Lebedyn beef at 9 months of age, reaching the lowest values (4.99%) in samples of Ukrainian Gray beef at 15 months of age. From 15 to 21 months of age, there is a gradual decrease in the amount of lysine from 6.50% to 6.40-6.04%. While at 3 months of age a statistically significant difference (p <0.05) was recorded between Simmental and Lebedyn breeds in the content of this amino acid, at 15 months, significant differences were found between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); at 21 months – between the Lebedyn and Simmental breeds (p <0.01) and between the Lebedyn and Ukrainian Gray breeds (p <0.01).

The shifts in histidine content, an essential amino acid for metabolic processes and animal growth, were more uniform in beef samples by breed. They gradually increased from 2.00-2.95% at three months to 3.45-4.28% at nine months, slowed slightly to 2.52-3.13% at 15 months, and stabilised at 3.10-3.68% at 21 months. The highest histidine concentration was observed in Ukrainian Grey beef samples at three months of age (2.95%), followed by Lebedyn beef samples at nine months of age (4.28%). The lowest concentration was observed in Ukrainian Grey beef samples at 15 months of age (2.52%). Statistically significant differences in histidine content were found between the Lebedyn and Simmental breeds (p <0.01) and between the Simmental and Ukrainian Grey breeds (p <0.01) at 3 months of age; between the Lebedyn and Simmental breeds (p <0.001), Lebedyn and Ukrainian Grey breeds (p <0.001) and Simmental and Ukrainian Grey breeds (p <0.001) at 15 months; and between the Lebedyn and Simmental breeds (p <0.05), Lebedyn and Ukrainian Grey breeds (p <0.01) and Simmental and Ukrainian Grey breeds (p<0.05) at 21 months. No statistically significant differences between breeds were found at 9 months of age.

Bulls showed a favourable increase in total threonine content, a priority in the use of food amino acids (key function), from 2.82-3.61% at 3 months to 4.53-5.02% at 9 months. From 9 to 15 months, the increase was less pronounced, decreasing to 2.88-4.06%. At 21 months, the enrichment rate increased further to 4.08-4.96%. Notably, Lebedyn beef samples had the highest amino acid content at 3 months (3.61%), 9 months (5.02%), and 21 months (4.96%). Against this background, the difference in threonine content between the Lebedyn and Simmental breeds was significant at 3 months (p <0.01); at 15 months, it was significant between the Lebedyn and Simmental breeds (p <0.001), the Lebedyn and Ukrainian Gray breeds (p <0.001) and the Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months, it was significant between the Simmental and Lebedyn breeds (p <0.001), the Simmental and Ukrainian Gray breeds (p <0.001) and the Ukrainian Gray and Lebedyn breeds (p <0.05).

A peculiar breed characteristic of the age dynamics of the content of the amino acid arginine in beef samples, which plays an important role in the processes of spermatogenesis and, above all, in achieving high gains, is its increase from 3.36-3.68% at 3 months to 4.45-5.77% at 15 months and a gradual decrease to 4.67-4.90% at 21 months Despite this, the highest arginine content was found in Lebedyn beef samples at 9 and 15 months of age (6.64% and 5.77% respectively), followed by Simmental beef at 21 months (4.90%). Furthermore, the difference in arginine content between 15-month-old Lebedyn and Simmental beef samples was statistically significant (p <0.05), as were the differences between Lebedyn and Ukrainian Grey beef samples (p <0.001) and between Simmental and Ukrainian Grey beef samples (p <0.001).

Considering the dynamics of valine formation, which supports normal nervous system function, we noted that breed clearly influenced its steady increase from 2.99-3.20% at 3 months to 3.24-3.77% at 21 months of age. The largest shift in valine content was 3.20% in samples of Ukrainian Gray beef at 3 months of age, 3.67% in samples of Lebedyn breed at 15 months of age, and 3.77% in samples of Simmental at 21 months of age. The breed had a greater effect on the increase in valine content in samples of Lebedyn beef at 15 months of age compared to Simmental (p <0.01) and Ukrainian Gray (p <0.001), Simmental breed relative to gray Ukrainian breed (p <0.001), at 21 months – Simmental breed relative to Lebedyn breed (p <0.05), Simmental breed relative to gray Ukrainian breed (p <0.001), Lebedyn breed relative to gray Ukrainian breed (p <0.05).

Despite this, methionine, an amino acid that promotes live weight and hair growth and prevents protein coagulation, had the lowest concentration among all identified amino acids. In addition, during the period of growing bulls of all breeds from 3 to 21 months of age, the methionine content in beef samples remained almost at the previously achieved level, from 1.02-1.17% at 3 months to 1.46-1.82% at 21 months, in contrast to 9 months of age, where its values again recovered moderately and were the highest – 1.28-1.99%. Against the background of the identified features, the maximum methionine content was recorded in Lebedyn beef samples at 3 and 9 months of age, respectively – 1.17 and 1.99%. Having the lowest values of methionine content in the

total structure of amino acids, a significant difference was achieved between breeds by this indicator, namely: at 9 months of age between the Lebedyn and Simmental breeds (p <0.01) and between the Simmental and Ukrainian Gray breeds (p<0.05); at 15 months - between the Lebedyn and Ukrainian Gray breeds (p <0.001) and between the Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months – between the Lebedyn and Simmental breeds (p <0.01) and between the Lebedyn and Ukrainian Gray breeds (p <0.001).

It is sufficient to note that the content of the amino acid isoleucine, which has a regulatory effect on the use of feed amino acids in the diet (a key function), and the age dynamics of breed increase in beef samples ranged from 2.44-2.86% at 3 months to 3.18-4.13% at 9 months of age. The period from 15 to 21 months of age is characterised by an increase in content from 3.06 to 3.46% in all breeds compared with the previous age period, except in the Ukrainian Grey breed at 21 months of age, where isoleucine content reached 4.19%. Statistical processing of the data on the content of this amino acid in beef samples revealed significant differences between breeds at 3 months between Lebedyn and Ukrainian Gray breeds (p <0.01), at 9 months – between Ukrainian Gray and Lebedyn breeds (p <0.01), Ukrainian Gray and Simmental breeds (p <0.05), at 15 months - between the Lebedyn and Simmental breeds (p <0.001), Simmental and Ukrainian Gray breeds (p <0.001), and Lebedyn and Ukrainian Gray breeds (p <0.001); at 21 months – between the Ukrainian Gray and Lebedyn breeds (p <0.001), Ukrainian Gray and Simmental breeds (p <0.001), and Simmental and Lebedyn breeds (p <0.01).

In turn, the content of the amino acid leucine, which is a vital component for the construction of plasma tissue proteins, showed a similar pattern to the amino acid isoleucine: a showed an age-related fluctuation in beef samples from 4.65-5.17% at 3 months to 5.18-7.09% at 9 months of age, while from 9 to 15 months there is a slowdown in its synthesis to 5.07-5.54% and a reverse increase in the content from 15 to 21 months of age – to 5.82-6.45%. The highest leucine content values in beef samples across age groups ranged from 5.17% (Lebedyn breed, 3 months) to 7.09%, 5.54%, and 6.45% (Ukrainian Grey breed, 9, 15, and 21 months).

Statistically significant differences in leucine content at 9 months of age were found between the Ukrainian Gray and Simmental breeds (p <0.05); at 15 months - between the Lebedyn and Simmental breeds (p <0.01), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); and at 21 months – between the Ukrainian Gray and Lebedyn breeds (p <0.01) and between the Ukrainian Gray and Simmental breeds (p <0.01).

Phenylalanine content, which is considered responsible for the synthesis of thyroxine and adrenaline hormones, did not significantly affect the peculiarities of its formation across different age periods. In particular, if from 3 to 21 months of age there was an activation of the synthesis of this amino acid in beef samples from 2.77-2.90% to 3.31-3.68%, then in the period from 9 to 15 months there is a slight decrease – from 3.20-3.73% to 2.82-3.54% against the background of almost the same level for all breed groups at the age of 21 months – 3.31-3.68%. When evaluating interbreed differences in phenylalanine content, a statistically significant difference was found at 15 months of age between the Lebedyn and Simmental breeds (p <0.01), Lebedyn and Ukrainian Gray breeds (p <0.001), and Simmental and Ukrainian Gray breeds (p <0.001); at 21 months – between the Lebedyn and Ukrainian Gray breeds (p <0.001), Lebedyn and Simmental breeds (p<0.05), and Simmental and Ukrainian Gray breeds (p <0.001).

A general pattern for the studied breeds is an increase in the amount of essential and nonessential amino acids in beef samples in the age dynamics of bulls' growth from 61.24-63.78% at 3 months to 72.48-76.09 at 21 months of age, including essential amino acids from 27.80-29.73% to 36.05-37.55%, and nonessential amino acids from 33.44-34.29% to 36.43-38.54% (Table 4).

A distinctive age-related feature is a clear trend toward an increase in the ratio of essential to nonessential amino acids, from 0.83-0.87 at 3 months to 1.00-1.02 at 15 months. Later, as the bulls age, there is a tendency for this ratio to decrease at 21 months compared to 15 months of age, with the exception of the Ukrainian Gray breeds. Instead, at 15 months of age, the total amount of amino acids was significantly lower in the Ukrainian Gray breed than in the Lebedyn (p <0.05) and Simmental (p <0.05) breeds. At the same age, the sum of essential amino acids was significantly lower in the Ukrainian Gray breed than in the Lebedyn (p <0.01) and Simmental (p <0.05) breeds, while the sum of nonessential amino acids was also lower in the Ukrainian Gray breed than in the Lebedyn (p <0.05) and Simmental (p <0.05) breeds. No, statistically significant interbreed differences in the amino acid ratio were found at any of the studied ages.

Table 4 Age-related changes in the formation of amino acids in the composition of carcass proteins of bulls of combined breeds.

Indicator	Age, months	Breed		
		L (% ± SD)	S (% ± SD)	UG (% ± SD)
Amino acid sum	3	63.78 ± 0.80[a]	61.24 ± 1.45[a]	63.59 ± 3.98[a]
	9	83.71 ± 6.33[a]	73.52 ± 6.52[a]	84.71 ± 1.48[a]
	15	72.23 ± 1.19[a]	70.72 ± 4.82[a]	60.34 ± 3.02[b]
	21	72.48 ± 2.49[a]	76.09 ± 4.50[a]	73.90 ± 2.69[a]
Including: essential	3	29.73 ± 0.74[a]	27.80 ± 0.35[a]	29.30 ± 2.16[a]
	9	42.70 ± 3.66[a]	36.68 ± 2.79[a]	41.96 ± 2.43[a]
	15	36.55 ± 0.18[a]	35.54 ± 2.43[a]	30.11 ± 1.00[b]
	21	36.05 ± 1.35[a]	37.55 ± 2.21[a]	37.43 ± 1.71[a]
non-essential	3	34.05 ± 0.07[a]	33.44 ± 1.11[a]	34.29 ± 1.82[a]
	9	41.01 ± 2.68[a]	36.84 ± 3.76[a]	42.75 ± 1.79[a]
	15	35.68 ± 1.04[a]	35.18 ± 2.39[a]	30.23 ± 2.02[b]
	21	36.43 ± 1.24[a]	38.54 ± 2.31[a]	36.47 ± 1.66[a]
Amino acid ratio	3	0.87 ± 0.02[a]	0.83 ± 0.02[a]	0.85 ± 0.02[a]
	9	1.04 ± 0.02[a]	1.00 ± 0.02[a]	0.99 ± 0.09[a]
	15	1.02 ± 0.03[a]	1.01 ± 0.01[a]	1.00 ± 0.03[a]
	21	0.99 ± 0.04[a]	0.97 ± 0.02[a]	1.03 ± 0.02[a]

Notes: Simmental (S), Lebedyn (L) and Ukrainian gray (UG); [a, b, c] – different superscript letters within the same age group indicate statistically significant differences between breeds.

The obtained experimental data confirmed the dependence previously identified by other researchers and made it possible to state with a high degree of probability that the content of amino acids in beef depends on both individual biotic and abiotic factors **[10]**, **[22]**, **[24]**, **[25]**, **[26]**, **[33]**. Differences among breeds within the study were statistically significant, likely due to breed-specific beef protein quality. Research into the patterns that govern amino acid composition in beef during ontogenesis is promising because it provides a scientific basis for understanding the role of breed, as well as breeding, feeding, and processing factors, in variability. Key measures include selecting breeds and lines of animals with high meat productivity and balanced muscle amino acid composition, determining the optimal slaughter age, improving feeding and housing conditions, and controlling meat processing and storage technologies. Particular attention is given to achieving the optimal ratio of essential to nonessential amino acids in muscle tissue, ensuring optimal protein, fat, and water content, and maintaining the physicochemical properties of meat during processing. Implementing these measures will help produce high-quality beef efficiently, meeting modern consumer and market requirements, and ensuring scientifically sound breeding and technological practices.

Limitations

The obtained results should be interpreted in light of the study's limitations. The volume of experimental material was limited by the available organisational and financial resources; consequently, the number of experimental series was limited. The main limitation of the study is the small number of animals within each breed × age group (n = 3), which limits statistical power and requires caution when interpreting non-significant differences. Nevertheless, the resulting sample adhered to established methodological approaches and was sufficiently representative for the planned statistical analysis. No comparison was made between the established patterns and the corresponding indicators of bulls of other breeds and age groups raised under similar feeding and housing conditions; this limits the generalizability of the results beyond the genotypes and age periods studied. Laboratory analyses were performed solely on Longissimus dorsi muscle samples shortly after collection, in accordance with standardised procedures. At the same time, the influence of post-slaughter biochemical processes, as well as the duration and conditions of sample storage, on specific parameters under study cannot be entirely ruled out. Further research should use a larger sample of animals, expand the range of breeds and ages of the bulls, and increase the number of experimental series.

CONCLUSION

The analyzed *Longissimus dorsi* muscle samples contained seven determined non-essential amino acids and nine essential amino acids, the content of which varies depending on age and breed; cysteine was not determined due to the lack of a standard solution, tryptophan was not determined due to limitations of the method used. During ontogeny, the total amount of amino acids in the *Longissimus dorsi* muscle changes: it gradually increases from three months of age, temporarily decreases between nine and 15 months of age, and then increases again by 21 months of age. This reflects the stages of age- and breed-related protein quality formation in muscle tissue. The observed changes in essential amino acid content and ratio to non-essential amino acids characterise age-related differences in nutritional properties of beef proteins. The results indicate breed-related differences in the amino acid profile of the *Longissimus dorsi* muscle at different ages and age-related variation in the studied indicators. These results also provide a scientific basis for improving beef protein quality by optimising the selection, feeding, and breeding of bulls.

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