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Formation of the biological value of beef protein depending on the age and breed of bulls

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ABSTRACT

The article substantiates the expediency of assessing the content of essential amino acids in the samples obtained during the controlled slaughter of bulls for the protein of chicken eggs as an effective means of improving the quality of the management processes for producing biologically complete products. To ascertain the biological value of beef, samples of the longest back muscle were obtained from bulls of six domestic dairy breeds (Black-and-White, Red Steppe, and Angler dairy breeds and Simmental, Lebedyn, and Gray Ukrainian dairy breeds of combined productivity) at the ages of 3, 6, 9, 12, 15, 18, and 21 months. The experiments were conducted using the ion-exchange liquid column chromatography method on an automatic amino acid analyser (T-339 M) manufactured by Microtechna (Czech Republic). It has been demonstrated that the biological value of meat from bulls of the studied breeds is limited during ontogeny, primarily due to age-related factors. As bulls grow older and gain weight, their meat proteins exhibit increased biological value, approaching the reference index of chicken egg proteins. The first peak in the average values of the amino acid index in beef of bulls of 12 months of age (0.89%) was followed by a consistent decrease to 15 months (0.68%) and a repeated increase in values in animals of 21 months of age (0.83%). This is mainly due to the rise in the scores for methionine by 0.16%, isoleucine by 0.16%, histidine by 0.42%, arginine by 0.18%, and threonine by 0.20%. The increase in the biological value of the remaining amino acid scores in the age trend of changes did not exceed 0.15%. The identified patterns indicate the presence of additional reserves in the near-term scenario, which can be utilised to ensure the production of high-grade beef while optimising the age parameters of slaughtering bulls of different productivity directions.

Keywords: amino acid score, age, breed, beef

INTRODUCTION

The significance of protein deficiency in human diets is increasing today. A lack of protein can result in many adverse effects, including a decline in metabolic function, an impairment of the immune system, and the malfunction of specific organs or systems. One potential solution is the development of animal agriculture, a strategy to alleviate protein deficiency **[1]**, **[2]**. Concurrently, the enhancement of production and the assurance of the quality and safety of meat, from its initial production to its final consumption, including beef, represents the fundamental instrument for its implementation **[3]**. Meat production, in general, and beef, in particular, depends on many factors, such as religious and cultural preferences, convenience, accessibility, etc. **[4]**. The term "beef" comes from the common Slavic word "govedo," which means "bull". Beef meat is an excellent source of lipids, carbohydrates, biologically active substances, and micro- and trace elements **[5]**, **[6]**, **[7]**. However, its most important component is high-quality protein, which provides the body with plastic material **[8]**. Protein consists of amino acids. All the essential amino acids contained in beef make it a complete and balanced protein

[9], the bioavailability of which depends on the proper organization of feeding, and housing conditions **[10]**, **[11]**, **[12]**.

Breed is one of the key factors influencing muscle tissue characteristics **[13]**, **[14]**, **[15]**. First of all, it affects the meat yield and the ratio of muscle, bone, and fat tissue. These components determine not only the quality of the meat but also its nutritional value. This is well illustrated in the study **[16]**. The researchers proved that the best indicators for the content of valine – by 7.4%, isoleucine – by 45.3% (p <0.001), leucine – by 15.2% (p <0.001), lysine – by 7.8%, threonine and phenylalanine + tyrosine by 6.5% (p <0.05) and 7.5% (p <0. 01) in 6-7-month-old bulls of the Charolais breed compared to Aberdeen-Angus and Black-and-White mixed breed, raised according to the technology of beef cattle breeding, compared to their counterparts of the Black-and-White breed. On the other hand, the amino acid score for most amino acids within all breeds exceeded 100%, indicating the high biological and nutritional value of veal. On the other hand, no significant differences were found between the breeds except for the histidine content when studying the characteristics of the amino acid profile of beef from Hungarian Grey and Holstein-Friesian bulls **[17]**. We cannot ignore the detailed studies **[18]**, which registered a significant difference in the content of essential amino acids in beef samples ranging from 30.16% in Montbéliard bulls to 34.50% in Menck-Anjou bulls and from 30.22% in Hereford bulls to 33.75% in Aberdeen Angus bulls, which was mainly due to the content of lysine – 7.85-8.73% and 7.76-8.75%, respectively. Age-related characteristics, which also affect the biological value of beef, have been well studied **[19]**, **[20]**. However, **[21]** found no significant differences in the amino acid content of beef due to the age of Qinchuan cattle.

Information on the content of amino acids in food products, including beef, can be used to determine their ability to meet human protein needs **[22]**. It is worth noting that with the development of the latest analytical methods for quality assessment, there is a growing interest in fundamental and applied research aimed at reviewing the role of amino acids in the formation of the biological value of livestock products, including beef, which is determined by chemical, biochemical and biological methods. At present, the most informative biochemical method is determining the amino acid number – the "score". Its fundamentally important essence lies in consistently comparing the concentration of a specific essential amino acid in a product with the content of the same amino acid in an ideal protein, which is taken as the amino acid composition of chicken egg white **[23]**, **[24]**. The proper implementation of this methodological assessment apparatus aims to increase the efficiency of quality management of final products during their industrial processing.

Over the past decade, Ukraine has significantly increased its understanding of the leading role of healthy eating in stimulating and activating the human body's defences through consuming nutritious products, especially beef. However, the production of this type of product is in a state of recovery. That is why one of the vectors for the development of this area in the context of protein deficiency is to produce high-protein meat products at the optimal age, to achieve scientifically sound pre-slaughter live weight of young animals, and to use breeds with increased genetic potential for increasing protein in carcasses. With the implementation of this task, the problem of vitamin nutrition can be completely solved, since the absorption of vitamins in the human body directly depends on the provision of biologically complete protein. Instead, the generalization of domestic literature sources that have begun to address this issue shows insufficient attention from scientists who only partially disclose it, encouraging the experimental development of appropriate recommendations.

Based on these considerations, the research aimed to determine the parameters that form beef protein's biological value depending on the bulls' age and breed.

Scientific Hypothesis

The breed and age of bulls can influence the formation of the biological integrity of the longest back muscle, which may deviate from the general trends of its formation in other breeds of the corresponding direction of productivity.

MATERIAL AND METHODOLOGY

Samples

During the growing period (from 30 days to 21 months of age), the experimental animals were fed diets of the same nutritional value, taking into account detailed feeding standards, which provided for 900-1000 g of average daily gain. The level of feeding during the growing period was high. It was designed to identify potential opportunities for increasing meat productivity and achieving a live weight of 550-650 kg by bulls at 18-21 months. The formation of meat productivity of bulls was evaluated at 3, 6, 9, 12, 15, 18, 21 months of age. The control slaughter was performed in the Kharkiv Meat Processing Plant, which met the requirements of DSTU 4673:2006: "Cattle for slaughter. Technical Conditions" **[25]**. The live weight of bulls formed into groups for slaughter corresponded to the average live weight of animals at the end of a certain age period. In order to determine the content of amino acids, samples of the longest muscle of the back were taken from three heads of each breed and of each age.

Chemicals

All chemicals were purchased from reputable brands on the market and met the highest analytical standards: alcohol solution of ninhydrin (grade A, chemically pure, Private Enterprise "Systema Optimum", Ukraine); Nessler's 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 "Khimlaborreactiv", Ukraine), ammonium sulfate, $(NH_4)_2SO_4$ (grade A, chemically pure, Private Joint Stock Company "SUMYKHIMPROM", Ukraine).

Animals, Plants and Biological Materials

The experiment was conducted in the production conditions of the basic farm of the Institute of Animal Science of the NAAS in the Kharkiv region, using purebred bulls of Black-and-White, Red Steppe, and Angler dairy breeds and Simmental, Lebedyn, and Gray Ukrainian dairy breeds of combined productivity. Three groups of 25 bulls each were formed and kept untethered in group sections of the same facility until they were 4 months old. After that, they were tethered until the end of the intensive growing period.

Instruments

The amino acid content was analyzed using ion-exchange liquid column chromatography **[26]**, **[27]**, **[28]** on an automatic amino acid analyzer T-339 M manufactured by Microtechna (Czech Republic) in 100 µL of hydrolyzate with the following sequence of phosphate-buffered amino acid eluates from the column: asparagine, threonine, serine, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine.

Laboratory Methods

The selected samples were examined in the production conditions of the laboratory for assessing the quality of feed and products of animal origin, part of the Testing Center of the Institute of Animal Husbandry of the National Academy of Agrarian Sciences of Ukraine certified for technical competence by the National Accreditation Agency of Ukraine, according to the requirements DSTU ISO/IEC 17025:2006 та DSTU EN ISO/IEC 17025:2019 as a basic organisation of the metrological service of the Ministry of Agrarian Policy and Food of Ukraine. The content of amino acids was determined by ion-exchange liquid column chromatography. The amino acid score, calculated according to the H. Mitchell and R. Block method, was considered an indicator of the biological value of beef protein. It shows the ratio of the content of an essential amino acid in the tested protein to its amount in the "ideal" protein **[29]**. The amino acid composition of chicken egg proteins (ideal protein) was taken as the standard for the biological value of protein. Additionally, methods generally accepted in experimental work and laboratory practice were used.

Description of the Experiment

Sample preparation: Samples of the longest back muscle were taken after primary processing of the carcasses, before the start of the cooling process. Samples from each carcass were cut out using a special boning knife. After that, they were freed from the connective tissue, placed in a sterile plastic film bag with a Zip-Lock fastener to prevent air access, and labelled. The samples were delivered to the laboratory in a cooler bag at a temperature of +6 ±2 °C. Protein hydrolysates were prepared in the laboratory to determine the amino acid content. The percentage of essential and nonessential amino acids is the sum of the latter's content; the concentration of each was determined by adding an alcohol solution of ninhydrin (pH 5.8) at 98 °C for 24 hours, followed by photometry of the stained samples. The acids cysteine and tryptophan content were not recorded in the absence of the corresponding standard solutions required for the construction of the calibration curve. The amino acid content was calculated as a percentage of the mass fraction of protein content in samples of the longest back muscle of the studied breeds. The mass fraction of protein was calculated using a conversion factor of 6.25 multiplied by the mass fraction of total nitrogen determined with Nessler's reagent after wet ashing the material in sulfuric acid by the methods specified in GOST 766-85 and expressed as a percentage of the mass fraction of the longest back muscle samples of bulls. Ammonium sulfate was used as a standard solution to prepare the calibration curve to determine total nitrogen content. The ratio of essential and nonessential amino acids is calculated by the amino acid index.

Number of samples analyzed: A total of 126 samples of the longest back muscle have been examined.

Number of repeated analyses: The experiments were repeated on six breeds, with the experimental data subjected to mathematical statistical analysis.

Number of experiment replication: The experiments were carried out in 7 age periods of bulls' growth with three samples taken from each breed, resulting in 21 repeated analyses.

Design of the experiment: A scientific and economic experiment was conducted in the first stage. The animals were provided with the feed of our production. Then we performed a controlled slaughter of bulls from six cattle breeds at the age of 3, 6, 9, 12, 15, 18, and 21 months, during which three samples of the longest back muscle were taken from each carcass of the corresponding breed and age at the level of 9-12 ribs. Subsequently, the samples were delivered to the laboratory to determine the amino acid content. The final stage involved summarising the results, subjecting them to statistical analysis, and testing the validity of the hypothesis.

Statistical Analysis

The obtained results within the studied breeds and relevant age periods were processed by methods of variation statistics using the software package for analysis of variance (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). Results are presented as mean ± standard error (x ±SE). Tukey's test was used to compare the difference in mean values between groups, where differences were considered statistically significant at p <0.05 for all data.

RESULTS AND DISCUSSION

Most scientific publications have convincingly demonstrated the significant advantage of using animal protein in the human diet, which is characterised by a high balance of essential and non-essential amino acids closest to human amino acid composition **[30]**. Beef contains a wide range of amino acids **[31]**, which influence its taste **[32]**, play a key role in its quality, depend directly on the breed factor **[33]** and are determined by the age of the animals **[34]**. They are one of the main components of meat **[35]**. However, Besung et al. **[36]** found no significant differences when comparing Bali and Wagyu beef. The picture is somewhat different for Japanese black cattle **[37]**. In this respect, the work of Nogi et al. **[38]** is interesting. The contradictions between the differences in amino acid content between breeds and the scientific justification for the time of slaughter became the basis for separate studies.

It is worth noting that the general pattern for all breeds is a slight age-related increase in the amino acid score of beef proteins from 3 to 6 months of age for lysine from 0.75 to 0.89% (+0.14%), methionine – from 0.36 to 0.47% (+0.11%), threonine – from 0.63 to 0.73% (+0.16%), isoleucine – from 0.39 to 0.45% (+0. 06%), leucine – from 0.53 to 0.58% (+0.05%), phenylalanine – from 0.51 to 0.59% (+0.08%), arginine – from 0.53 to 0.58% (+0.05%), histidine – from 1.06 to 1.22% (+0. 16%), valine – from 0.42 to 0.49% (0.07%) against the background of an increase in the total score – from 5.18 to 5.99% (+0.81%) and amino acid index – from 0.58 to 0.67% (0.09%). The results of the experiment are shown in Table 1.

Breed and age differences in beef in most amino acid scores were not statistically significant. Nevertheless, the difference between Lebedyn and Angler, Gray Ukrainian, Simmental, and Black-and-White breeds at 3 months of age became significant at p <0.05, as well as the difference between Gray Ukrainian, Angler and Simmental breeds at p <0.05. With the increase in the age of bulls to 6 months, the most significant changes were noted in the threonine amino acid between the Black-and-White and Red Steppe, Angler, Lebedyn, and Simmental breeds (p <0.01). The highest statistical significance (p <0.001) was achieved for the essential amino acid histidine rate between the Black-and-White, Simmental, Angler, and Lebedyn breeds. The data obtained extend the results regarding the impact of breed factor on beef quality **[39]**.

A comparison of the average data for beef samples with the standard indicates an increase in the total amount of amino acids, with a statistically significant difference between the Angler and Black-and-White, Simmental breeds (p <0.05) and between the Black-and-White and Simmental breeds (p <0.01). The amino acid index values calculated for samples taken from bulls at 3 and 6 months did not reveal any significant differences between the breeds.

The formation of the fullness of proteins of the pulp of bull carcasses in the period from 9 to 12 months of age was accompanied by an increase in the scores for methionine from 0.50 to 0.65% (+0.15%), isoleucine - from 0.46 to 0.61% (+0.15%), leucine – from 0.62 to 0.67% (+0.05%), phenylalanine – from 0.61 to 70% (+0.09%), arginine – from 0.75 to 1.03% (+0.28%), histidine – from 1.54 to 1.74% (+0.20%), valine – from 0.51 to 0.59% (0.08%) against the background of a decrease in the average values of amino acid scores for lysine – from 1.05 to 1.04% (-0.01%) and threonine – from 0.97 to 0.61% (-0.36%). Nevertheless, the total sum of the scores of all essential amino acids increased by 0.80% during this age period, which cannot be reliably stated about the amino acid index, which increased by only 0.09%.

At the same time, the difference in the value of the histidine amino acid in the beef of bulls of 9 months of age between the Lebedyn and Black-and-White, Simmental, Angler, Gray Ukrainian breeds was the most prominent and statistically significant (p <0.01) than the rest of the parameters of its evaluation, where it was p <0.05 for the amino acid lysine between Lebedyn and Angler breeds, p <0.05 for isoleucine between Gray Ukrainian and Simmental, Black-and-White breeds, p <0.05 for the leucine between Gray Ukrainian and Simmental, Black-and-White breeds.

Table 1 Formation of the fullness of carcass pulp proteins in ontogeny chicken egg protein, %.

Chicken egg protein (standard)	Breed						On average by breeds
	Lebedyn	Black-and-White	Red Steppe	Simmental	Angler	Gray Ukrainian	
3 months							
Lysine – 6.9	0.72 ±0.01^{a}	0.75 ±0.02^{a}	0.73 ±0.01^{a}	0.79 ±0.03^{a}	0.78 ±0.02^{a}	0.73 ±0.01^{a}	0.75 ±0.02
Methionine – 3.3	0.35 ±0.04^{a}	0.30 ±0.03^{a}	0.38 ±0.05^{a}	0.31 ±0.03^{a}	0.44 ±0.07^{a}	0.35 ±0.04^{a}	0.36 ±0.04
Threonine – 5.0	0.72 ±0.02^{a}	0.55 ±0.01^{b}	0.68 ±0.02^{ab}	0.57 ±0.03^{b}	0.64 ±0.02^{b}	0.64 ±0.02^{b}	0.63 ±0.02
Isoleucine – 6.9	0.41 ±0.01^{a}	0.37 ±0.02^{a}	0.40 ±0.01^{a}	0.36 ±0.03^{a}	0.45 ±0.03^{a}	0.35 ±0.02^{a}	0.39 ±0.02
Leucine – 9.4	0.55 ±0.03^{a}	0.49 ±0.02^{a}	0.53 ±0.03^{a}	0.49 ±0.02^{a}	0.58 ±0.04^{a}	0.51 ±0.03^{a}	0.53 ±0.03
Phenylalanine – 5.8	0.49 ±0.02^{a}	0.52 ±0.03^{a}	0.52 ±0.02^{a}	0.48 ±0.01^{a}	0.55 ±0.03^{a}	0.50 ±0.01^{a}	0.51 ±0.02
Arginine – 6.7	0.50 ±0.03^{a}	0.54 ±0.04^{a}	0.52 ±0.04^{a}	0.54 ±0.02^{a}	0.54 ±0.03^{a}	0.55 ±0.04^{a}	0.53 ±0.03
Histidine – 2.4	1.10 ±0.06^{ab}	1.08 ±0.05^{ab}	1.16 ±0.02^{ab}	0.83 ±0.01^{a}	0.97 ±0.02^{a}	1.23 ±0.07^{b}	1.06 ±0.04
Valine – 7.4	0.41 ±0.02^{a}	0.42 ±0.01^{a}	0.43 ±0.03^{a}	0.40 ±0.04^{a}	0.43 ±0.06^{a}	0.43 ±0.04^{a}	0.42 ±0.03
Total amount of amino acids – 9.0	5.25 ±0.07^{a}	5.02 ±0.05^{a}	5.35 ±0.06^{a}	4.77 ±0.02^{a}	5.38 ±0.08^{a}	5.29 ±0.04^{a}	5.18 ±0.05
Amino acid index – 1.0	0.58 ±0.01^{a}	0.56 ±0.02^{a}	0.59 ±0.02^{a}	0.53 ±0.03^{a}	0.60 ±0.04^{a}	0.59 ±0.03^{a}	0.58 ±0.03
6 months							
Lysine – 6.9	0.92 ±0.03^{a}	0.88 ±0.01^{a}	0.92 ±0.03^{a}	0.85 ±0.02^{a}	0.91 ±0.03^{a}	0.84 ±0.02^{a}	0.89 ±0.02
Methionine – 3.3	0.43 ±0.06^{a}	0.54 ±0.01^{a}	0.51 ±0.09^{a}	0.44 ±0.07^{a}	0.42 ±0.06^{a}	0.48 ±0.08^{a}	0.47 ±0.06
Threonine – 5.0	0.69 ±0.03^{a}	0.85 ±0.02^{b}	0.70 ±0.01^{a}	0.66 ±0.04^{a}	0.70 ±0.02^{a}	0.80 ±0.03^{ab}	0.73 ±0.02
Isoleucine – 6.9	0.45 ±0.03^{a}	0.41 ±0.01^{a}	0.45 ±0.03^{a}	0.44 ±0.02^{a}	0.45 ±0.03^{a}	0.51 ±0.04^{a}	0.45 ±0.03
Leucine – 9.4	0.58 ±0.02^{a}	0.56 ±0.03^{a}	0.58 ±0.03^{a}	0.58 ±0.03^{a}	0.56 ±0.02^{a}	0.61 ±0.05^{a}	0.58 ±0.03
Phenylalanine – 5.8	0.57 ±0.04^{a}	0.60 ±0.03^{a}	0.55 ±0.02^{a}	0.55 ±0.02^{a}	0.69 ±0.05^{a}	0.59 ±0.03^{a}	0.59 ±0.03
Arginine – 6.7	0.61 ±0.01^{a}	0.59 ±0.01^{a}	0.55 ±0.03^{a}	0.55 ±0.03^{a}	0.57 ±0.02^{a}	0.60 ±0.03^{a}	0.58 ±0.02
Histidine – 2.4	1.14 ±0.02^{a}	1.49 ±0.01^{b}	1.11 ±0.02^{ab}	1.34 ±0.03^{a}	1.24 ±0.02^{a}	0.98 ±0.04^{ab}	1.22 ±0.02
Valine – 7.4	0.45 ±0.02^{a}	0.54 ±0.01^{a}	0.46 ±0.05^{a}	0.45 ±0.03^{a}	0.48 ±0.04^{a}	0.54 ±0.06^{a}	0.49 ±0.04
Total amount of amino acids – 9.0	5.84 ±0.05^{abc}	6.46 ±0.07^{a}	5.83 ±0.04^{abc}	5.86 ±0.05^{b}	6.02 ±0.04^{c}	5.95 ±0.07^{adc}	5.99 ±0.05
Amino acid index – 1.0	0.65 ±0.03^{a}	0.72 ±0.04^{a}	0.65 ±0.03^{a}	0.65 ±0.03^{a}	0.67 ±0.02^{a}	0.66 ±0.02^{a}	0.67 ±0.03
9 months							
Lysine – 6.9	1.15 ±0.06^{a}	1.14 ±0.05^{ab}	1.01 ±0.03^{ab}	0.99 ±0.04^{ab}	0.94 ±0.02^{b}	1.07 ±0.04^{ab}	1.05 ±0.04
Methionine – 3.3	0.60 ±0.05^{a}	0.52 ±0.04^{a}	0.51 ±0.05^{a}	0.39 ±0.07^{a}	0.39 ±0.07^{a}	0.57 ±0.02^{a}	0.50 ±0.05
Threonine – 5.0	1.00 ±0.02^{a}	1.02 ±0.02^{a}	1.02 ±0.02^{a}	0.91 ±0.03^{a}	0.96 ±0.04^{a}	0.92 ±0.03^{a}	0.97 ±0.03
Isoleucine – 6.9	0.46 ±0.02^{ab}	0.35 ±0.01^{a}	0.34 ±0.01^{ab}	0.47 ±0.02^{a}	0.54 ±0.03^{ab}	0.60 ±0.04^{b}	0.46 ±0.02
Leucine – 9.4	0.70 ±0.04^{ab}	0.52 ±0.03^{ac}	0.61 ±0.04^{a}	0.55 ±0.02^{b}	0.59 ±0.03^{a}	0.75 ±0.05^{a}	0.62 ±0.04
Phenylalanine – 5.8	0.55 ±0.02^{a}	0.62 ±0.04^{a}	0.65 ±0.03^{a}	0.59 ±0.04^{a}	0.59 ±0.03^{a}	0.64 ±0.03^{a}	0.61 ±0.03
Arginine – 6.7	0.99 ±0.02^{a}	1.01 ±0.04^{a}	0.99 ±0.02^{a}	0.79 ±0.03^{a}	0.82 ±0.03	0.91 ±0.02	0.75 ±0.03
Histidine – 2.4	1.78 ±0.04^{a}	1.63 ±0.03^{b}	1.67 ±0.03^{ab}	1.43 ±0.02^{b}	1,31 ±0.01^{b}	1.44 ±0.02^{b}	1.54 ±0.03
Valine – 7.4	0.53 ±0.01^{a}	0.53 ±0.01^{a}	0.53 ±0.01^{a}	0.47 ±0.02^{a}	0.48 ±0.02^{a}	0.49 ±0.02^{a}	0.51 ±0.02
Total amount of amino acids – 9.0	7.76 ±0.02^{a}	7.34 ±0.03^{bc}	7.33 ±0.03^{b}	6.59 ±0.04^{b}	6.62 ±0.05^{b}	7.39 ±0.02^{b}	7.17 ±0.03
Amino acid index – 1.0	0.86 ±0.02^{a}	0.82 ±0.03^{ab}	0.81 ±0.02^{ab}	0.73 ±0.03^{b}	0.74 ±0.03^{b}	0.82 ±0.03^{ab}	0.80 ±0.03
12 months							
Lysine – 6.9	1.06 ±0.04^{ab}	0.98 ±0.03^{b}	0.91 ±0.02^{b}	1.17 ±0.03^{ac}	1.14 ±0.03^{c}	1.00 ±0.02^{b}	1.04 ±0.03
Methionine – 3.3	0.68 ±0.06^{a}	0.60 ±0.05^{a}	0.61 ±0.04^{a}	0.64 ±0.03^{a}	0.64 ±0.03^{a}	0.74 ±0.08^{a}	0.65 ±0.05
Threonine – 5.0	0.92 ±0.03^{ab}	0.90 ±0.03^{b}	1.02 ±0.04^{ab}	1.04 ±0.04^{a}	0.98 ±0.03^{ab}	0.81 ±0.02^{b}	0.61 ±0.03
Isoleucine – 6.9	0.63 ±0.03^{a}	0.65 ±0.02^{a}	0.54 ±0.01^{a}	0.64 ±0.03^{a}	0.59 ±0.02^{a}	0.61 ±0.02^{a}	0.61 ±0.02
Leucine – 9.4	0.63 ±0.03^{a}	0.62 ±0.03^{a}	0.69 ±0.04^{a}	0.67 ±0.02^{a}	0.63 ±0.03^{a}	0.75 ±0.04^{a}	0.67 ±0.03
Phenylalanine – 5.8	0.72 ±0.03^{a}	0.67 ±0.02^{a}	0.66 ±0.02^{a}	0.72 ±0.04^{a}	0.77 ±0.05^{a}	0.64 ±0.02^{a}	0.70 ±0.03
Arginine – 6.7	1.02 ±0.04^{a}	0.98 ±0.03^{a}	0.94±0.02^{a}	1.11 ±0.05^{a}	1.07 ±0.04^{a}	1.05 ±0.03^{a}	1.03 ±0.04
Histidine – 2.4	1.74 ±0.04^{ab}	1.85 ±0.05^{ab}	1.45 ±0.02^{b}	1.83 ±0.03^{a}	1.71 ±0.02^{b}	1.85 ±0.04^{ab}	1.74 ±0.03
Valine – 7.4	0.60 ±0.02^{a}	0.60 ±0.02^{a}	0.54 ±0.03^{a}	0.60 ±0.04^{a}	0.62 ±0.04^{a}	0.60 ±0.04^{a}	0.59 ±0.03
Total amount of amino acids – 9.0	8.00 ±0.05^{a}	7.81 ±0.04^{a}	7.36 ±0.02^{a}	8.42 ±0.03^{b}	8.15 ±0.02^{c}	8.05 ±0.04^{ac}	7.97 ±0.03
Amino acid index – 1.0	0.89 ±0.01^{abc}	0.87 ±0.01^{a}	0.82 ±0.02^{a}	0.94 ±0.03^{a}	0.91 ±0.01^{bc}	0.89 ±0.02^{abc}	0.89 ±0.02

A distinctive feature of beef samples by the total sum of amino acids scores is a statistically significant increase in its values between Lebedyn and Gray Ukrainian, Black-and-White, Red Steppe, Angler, and Simmental breeds at p <0.001 in all cases of comparison and the constancy of the amino acid index values between Lebedyn and Angler breeds (p <0.05), Simmental breed (p <0.05). A similar pattern of changes has been reported **[40]**.

Table 1 Cont.

Chicken egg protein (standard)	Breed						On average by breeds
	Lebedyn	Black-and-White	Red Steppe	Simmental	Angler	Gray Ukrainian	
15 months							
Lysine – 6.9	0.94 ±0.02^{a}	0.95 ±0.01^{a}	0.87 ±0.03^{ab}	0.83 ±0.03^{b}	0.87 ±0.03^{ab}	0.72 ±0.04^{b}	0.86 ±0.03
Methionine – 3.3	0.51 ±0.03^{a}	0.65 ±0.04^{b}	0.48 ±0.02^{a}	0.51 ±0.03^{a}	0.47 ±0.01^{a}	0.38 ±0.04^{a}	0.50 ±0.04
Threonine – 5.0	0.81 ±0.04^{ab}	0.85 ±0.04^{a}	0.71 ±0.03^{b}	0.73 ±0.02^{ab}	0.71 ±0.02^{ab}	0.58 ±0.01^{b}	0.73 ±0.03
Isoleucine – 6.9	0.45 ±0.02^{a}	0.41 ±0.02^{a}	0.45 ±0.02^{a}	0.51±0.04^{a}	0.41 ±0.02^{a}	0.41 ±0.02^{a}	0.44 ±0.02
Leucine – 9.4	0.54 ±0.02^{a}	0.53 ±0.03^{a}	0.55 ±0.02^{a}	0.56 ±0.04^{a}	0.53 ±0.03^{a}	0.48 ±0.02^{a}	0.53 ±0.03
Phenylalanine – 5.8	0.61 ±0.03^{a}	0.59 ±0.01^{a}	0.43 ±0.02^{b}	0.60 ±0.03^{a}	0.58 ±0.01^{ab}	0.49 ±0.02^{b}	0.55 ±0.02
Arginine – 6.7	0.86 ±0.02^{a}	0.84 ±0.02^{a}	0.82 ±0.01^{a}	0.82 ±0.01^{a}	0.80 ±0.02^{a}	0.66 ±0.03^{a}	0.80 ±0.02
Histidine – 2.4	1.30 ±0.02^{ab}	1.27 ±0.02^{ab}	1.17 ±0.01^{b}	1.37 ±0.03^{a}	1.16 ±0.01^{b}	1.05 ±0.01^{b}	1.22 ±0.02
Valine – 7.4	0.50 ±0.01^{abc}	0.55 ±0.02^{ac}	0.47 ±0.01^{bc}	0.47 ±0.01^{bc}	0.41 ±0.01^{c}	0.39 ±0.02^{ab}	0.47 ±0.01
Total amount of amino acids – 9.0	6.52 ±0.03^{c}	6,64 ±0.02^{ac}	5.95 ±0.02^{b}	6.40 ±0.01^{bc}	5.94 ±0.04^{bc}	5.16 ±0.03^{b}	6.10 ±0.02
Amino acid index – 1.0	0.72 ±0.02^{a}	0.74 ±0.03^{ab}	0.66 ±0.01^{a}	0.71 ±0.03^{ab}	0.66 ±0.01^{ab}	0.57 ±0.01^{b}	0.68 ±0.02
18 months							
Lysine – 6.9	0.89 ±0.02^{ab}	0.86±0.03^{a}	0.84 ±0.01^{ac}	0.94 ±0.04^{ab}	0.92 ±0.03^{ab}	0.98 ±0.01^{b}	0.91 ±0.02
Methionine – 3.3	0.60 ±0.03^{a}	0.54 ±0.04^{a}	0.52 ±0.01^{a}	0.52 ±0.01^{a}	0.59 ±0.03^{a}	0.60 ±0.03^{a}	0.56 ±0.03
Threonine – 5.0	0.80 ±0.03^{ab}	0.75 ±0.02^{a}	0.72 ±0.02^{a}	0.82 ±0.04^{ab}	0.77 ±0.02^{ab}	0.91 ±0.05^{b}	0.80 ±0.03
Isoleucine – 6.9	0.52 ±0.01^{a}	0.50 ±0.02^{a}	0.46 ±0.03^{a}	0.50 ±0.01^{a}	0.51 ±0.01^{a}	0.56 ±0.03^{a}	0.51 ±0.02
Leucine – 9.4	0.57 ±0.02^{a}	0.54 ±0.01^{a}	0.53 ±0.01^{a}	0.58 ±0.02^{ab}	0.59 ±0.02^{ab}	0.68 ±0.03^{b}	0.58 ±0.02
Phenylalanine – 5.8	0.51 ±0.02^{ab}	0.47 ±0.03^{a}	0.50 ±0.01^{a}	0.55 ±0.02^{ab}	0.55 ±0.02^{ab}	0.60 ±0.03^{b}	0.53 ±0.02
Arginine – 6.7	0.69 ±0.01^{a}	0.60 ±0.01^{a}	0.78 ±0.02^{ab}	0.64 ±0.03^{a}	0.77 ±0.02^{ab}	0.83 ±0.04^{b}	0.72 ±0.02
Histidine – 2.4	1.45 ±0.02^{a}	1.40 ±0.02^{a}	1.33 ±0.01^{b}	1.50 ±0.03^{a}	1.46 ±0.02^{a}	1.50 ±0.03^{a}	1.44 ±0.02
Valine – 7.4	0.45 ±0.01^{a}	0.43 ±0.01^{a}	0.43 ±0.01^{a}	0.46 ±0.02^{a}	0.45 ±0.02^{a}	0.51 ±0.04^{a}	0.46 ±0.02
Total amount of amino acids – 9.0	6.46 ±0.05^{ac}	6.03 ±0.03^{a}	6.10 ±0.02^{a}	6.51 ±0.04^{c}	6.59 ±0.06^{a}	7.15 ±0.02^{b}	6.47 ±0.04
Amino acid index – 1.0	0.72 ±0.02^{ab}	0.67 ±0.01^{a}	0.68 ±0.01^{a}	0.72 ±0.02^{ab}	0.73 ±0.02^{ab}	0.80 ±0.03^{b}	0.72 ±0.02
21 months							
Lysine – 6.9	0.93 ±0.03^{a}	0.95 ±0.02^{a}	0.89 ±0.02^{a}	0.88 ±0.02^{a}	0.86 ±0.01^{a}	0.88 ±0.02^{a}	0.90 ±0.02
Methionine – 3.3	0.55 ±0.02^{abc}	0.58 ±0.03^{c}	0.59 ±0.03^{b}	0.48 ±0.02^{bc}	0.45 ±0.01^{a}	0.44 ±0.01^{ab}	0.52 ±0.02
Threonine – 5.0	0.82 ±0.01^{a}	0.83 ±0.01^{a}	0.67 ±0.03^{a}	0.99 ±0.04^{b}	0.84 ±0.01^{b}	0.85 ±0.02^{b}	0.83 ±0.02
Isoleucine – 6.9	0.44 ±0.01^{a}	0.57 ±0.02^{ab}	0.55 ±0.02^{ab}	0.50 ±0.01^{a}	0.64 ±0.03^{ab}	0.61 ±0.03^{b}	0.55 ±0.02
Leucine – 9.4	0.62 ±0.03^{ab}	0.54 ±0.04^{a}	0.56 ±0.04^{a}	0.62 ±0.03^{ab}	0.60 ±0.02^{ab}	0.69 ±0.02^{b}	0.61 ±0.03
Phenylalanine – 5.8	0.63 ±0.02^{ab}	0.68 ±0.02^{a}	0.64 ±0.01^{ab}	0.62 ±0.02^{ab}	0.57 ±0.02^{b}	0.57 ±0.02^{b}	0.62 ±0.02
Arginine – 6.7	0.70 ±0.01^{a}	0.68 ±0.02^{a}	0.71 ±0.01^{a}	0.73 ±0.03^{a}	0.72 ±0.03^{a}	0.71 ±0.01^{a}	0.71 ±0.02
Histidine – 2.4	1.29 ±0.02^{a}	1.53 ±0.04^{ab}	1.58 ±0.03^{b}	1.42 ±0.02^{a}	1.52 ±0.03^{ab}	1.53 ±0.02^{b}	1.48 ±0.03
Valine – 7.4	0.46 ±0.02^{a}	0.46 ±0.02^{a}	0.50 ±0.03^{a}	0.51 ±0.03^{a}	0.44 ±0.02^{a}	0.44 ±0.02^{a}	0.47 ±0.02
Total amount of amino acids – 9.0	7.16 ±0.03^{b}	7.58 ±0.04^{a}	7.43 ±0.03^{b}	7.50 ±0.02^{a}	7.38 ±0.03^{abc}	7.47 ±0.02^{abc}	7.42 ±0.03
Amino acid index – 1.0	0.80 ±0.02^{a}	0.84 ±0.02^{a}	0.83 ±0.01^{a}	0.83 ±0.01^{a}	0.82 ±0.02^{a}	0.83 ±0.02^{a}	0.83 ±0.02

Note: Values are means ±SE; n = 3, different letters indicate significant differences between groups within each row by Tukey's test.

Increase in the amino acid scores for lysine in beef samples from bulls aged 12 months of Simmental and Gray Ukrainian breeds (p <0.01), Black-and-White (p <0.01), Red Steppe (p <0.01), Angler and Gray Ukrainian (p <0.05), Black-and-White (p <0.05), Red Steppe (p <0.01); for threonine – Simmental and Black-and-White breeds (p <0.05), Gray Ukrainian (p <0.01); for histidine – Simmental and Angler (p <0.05), Red Steppe (p <0.001), resulted in a significant increase in the total amount of scores: between Simmental and Angler (p <0.01), Gray Ukrainian (p <0.01), Lebedyn (p <0.01), Black-and-White (p <0.01), Red Steppe (p <0.001), between Angler and Lebedyn (p <0.05), Red Steppe (p <0.001), Black-and-White (p <0.01) and between Simmental and Red Steppe (p <0.05) breeds increased as a result of higher intensity and efficiency of protein synthesis in the muscle tissue of bulls of these breeds.

In turn, the quantitative indicators of amino acid scores in the period from 15 to 18 months of age were also determined by the breed of bulls and varied with age: increased for lysine from 0.86 to 0.91% (+0.05%), methionine – from 0.50 to 0.56% (+0. 06%), threonine – from 0.73 to 0.80% (+0.07), isoleucine – from 0.44 to 0.51% (+0.07%), histidine from 1.22 to 1.44% (+0.22%) and, conversely, decreased for leucine – from 0. 53 to 0.58% (-0.05%), valine – from 0.47 to 0.46% (-0.01%), phenylalanine – from 0.55 to 0.53% (-0.02%); arginine – from 0.80 to 0.72% (-0.08%). The research is consistent with **[41]**.

The breed of bulls also had a decisive influence on the differences in the values of the scores of individual amino acids in the carcass flesh in ontogeny. So far, at 15 months of age, a statistically significant difference in the rate of the amino acid lysine has been proven between the Black-and-White and Simmental breeds (p <0.05), between the Lebedyn and Simmental (p <0.05), and the Gray Ukrainian breeds (p <0.01); amino acid methionine – between Black-and-White and Simmental (p <0.05), Lebedyn (p <0.05), Red Steppe (p <0.05), Angler (p <0.05), Gray Ukrainian (p <0.01); threonine – between Black-and-White and Red Steppe (p <0.05), Gray Ukrainian (p <0.01); phenylalanine – between Simmental and Gray Ukrainian (p <0.05), Red Steppe (p <0.01), Black-and-White and Gray Ukrainian (p <0.05), Red Steppe (p <0.01), between Lebedyn and Red Steppe, Gray Ukrainian (p <0.01); histidine – between Simmental and Red Steppe, Angler, Gray Ukrainian (p <0.01), Lebedyn and Red Steppe, Gray Ukrainian (p <0.01); valine – between Black-and-White and Red Steppe, Simmental (p <0.05), Angler and Gray Ukrainian (p <0.01).

Notably, the main trend in the formation of the sum of the majority of amino acid scores related to the breed of the experimental groups was saved, and some of them reached high statistical significance. In particular, between Black-and-White and Simmental, Red Steppe, Angler, and Gray Ukrainian (p <0.001), between Lebedinsky and Red Steppe, Gray Ukrainian (p <0.001). Despite this, the highest values of the amino acid index were inherent in samples taken from bulls of the Black-and-White breed, which exceeded the Gray Ukrainian breed at the level of p <0.01. Lebedyn and Red Steppe breeds dominated the Gray Ukrainian breed by this indicator, at p <0.01 in both cases of comparison. The identified differences between breeds and age periods of bulls complement the materials obtained in **[42]**.

It is worth noting that the maximum amplitude of fluctuations in the values of amino acid scores for lysine in the studied samples of beef from 18-month-old bulls allowed to prove a significant difference between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.001); threonine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.05); leucine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe (p <0.05), between Gray Ukrainian and Lebedyn (p <0.05), Black-and-White (p <0.05), Red Steppe breeds (p <0.05); phenylalanine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.05); arginine - between Gray Ukrainian and Lebedyn (p <0.05), Simmental (p <0.05), Black-and-White breeds (p <0.01); histidine – between Gray Ukrainian and Red Steppe (p <0.01), Simmental and Red Steppe (p <0.01), Angler and Red Steppe (p <0.01), Lebedyn and Red Steppe (p <0.01), Black-and-White and Red Steppe (p <0.01).

The breed of bulls largely determined the increase in the total amount of amino acid scores between the Ukrainian Grey and Angler (p <0.001), Simmental (p <0.001), Lebedyn (p <0.001), Red Steppe (p <0.001), Black-and-White (p <0.001) breeds, as well as between Simmental and Red Steppe (p <0.001), Black-and-White (p <0.001) breeds. Despite the statistically significant differences between breeds for the corresponding indicator, the highest biological value is inherent in the samples of Gray Ukrainian beef, which in terms of amino acid index prevailed over both Red Steppe and Black-and-White breeds at an identical level of significance p <0.05. The dependence of the amino acid composition of beef on the breed factor has been established **[43]**.

Regarding the difference in the values of amino acid scores in the period from 15 to 21 months of age, an increase in lysine from 0.86 to 0.90% (+0.04%), methionine from 0.50 to 0.52% (+0.02%), threonine from 0.73 to 0.83% (+0.10%), isoleucine – from 0.44 to 0.55% (+0.11%), leucine – from 0.53 to 0.61% (+0.08%), phenylalanine – from 0.55 to 0.62% (+0.07%), histidine – from 1.22 to 1.48% (+0.26%) with a simultaneous decrease in arginine – from 0.80 to 0.71% (-0.09%) is observed. Beef contained the same score of the amino acid valine compared to chicken egg protein.

A characteristic interbreed difference in the rate of the amino acid methionine is inherent in the beef samples from 21-month-old bulls. However, the results were not significant or stable, reaching the highest level of significance between the Red Steppe and Simmental breeds up to p <0.05, Angler – p <0.05, Gray Ukrainian – p <0.01, as well as between Black-and-White and Angler – p <0.05, and Gray Ukrainian – p <0.05. The leading position in the threonine amino acid score was occupied by Simmental beef samples, where its values were higher than those of Ukrainian Grey, Angler, Black-and-White, and Lebedyn breeds at the same level of significance (p <0.05), and the same indicator in Simmental samples was also better than in samples of the Red Steppe breed (p <0.01). In addition, by the values of the amino acid isoleucine, a significant difference was found only between the Gray Ukrainian and Simmental (p <0.05), Lebedyn (p <0.01) breeds; by the amino acid leucine - between the Gray Ukrainian and Red Steppe (p <0.05), Black-and-White (p <0.05); in terms of the amino acid phenylalanine – between Black-and-White, Angler and Ukrainian Grey breeds (p <0.05); in terms of the amino acid histidine – between Red Steppe and Simmental (p <0.05), Lebedyn breeds (p <0.01), Ukrainian Grey and Simmental (p <0.05), Lebedyn breeds (p <0.01). The identified features ultimately determined the changes in the value of the total sum of all amino acid scores. Comparing the biological value of beef scores with chicken egg protein, it can be argued that in the final period of the experiment, according to the total amount of points obtained, samples of the Black-and-White breed significantly exceeded samples of the Red Steppe (p <0.05), Angler (p <0.05), Lebedyn (p <0.01), Simmental and Angler (p <0.05), and Lebedyn (p <0.001) breeds. A higher total score of samples of the Gray Ukrainian breed caused significant differences in beef samples from Lebedyn bulls (p <0.01). The synchronous pattern of increasing the total protein complex of short-chain amino acids in the samples of Angler breed beef was also observed concerning the samples of the Lebedyn (p <0.05). However, it should be emphasised that there were no statistical differences between the values of the amino acid index in any of the breeds. Our results are consistent with those of the research **[44]**.

Beef obtained from bulls of 21 months of age was characterised by a rather high biological value compared to 3 months of age due to an increase in the rates of amino acids by methionine by 0.16%, isoleucine by 0.16%, histidine by 0.42%, arginine – by 0.18%, threonine – by 0.20%, valine – by 0.05%, leucine – by 0.08%, phenylalanine – by 0.11% and lysine – by 0.15%, which, in turn, ensures its potential ability to be sold in the retail network without restrictions of higher quality.

The revealed nonlinear trend in changes in the beef protein completeness index and the total amount of amino acids is determined by the peculiarities of the formation of the fractional composition of proteins, and muscle and connective tissue at different stages of ontogenesis.

Thus, based on the results obtained, the rational slaughter age for bulls of the breeds studied can be considered to be 18-21 months, while for veal production it is 12 months, but the data obtained should first be consistent with the regulated norm of live weight of animals for the corresponding age period.

The dependence of the biological value of meat on the age of animals is consistent with the findings of Kim et al. **[13]** and Kodani et al. **[14]**. In addition to age, the biological value of beef is determined by the content of certain amino acids in different anatomical parts of bull carcasses. In particular, Cho et al. **[45]**, when analysing samples taken from different parts of the carcasses of 10 Hanwoo bulls at 24 months of age, the highest content of glutamine and alanine was found, and slightly lower content of arginine, phenylalanine, and lysine. This indicates the high biological value of beef regardless of the sampling location. Additional examples of meat quality formation depending on the animal breed can be found in work Bischof et al. **[15]**. A similar result was obtained in a study Vopálenský et al. **[46]** conducted on eight groups of beef cattle breeds of the same age, where scientists demonstrated a number of interbreed differences between the values of non-essential and essential amino acids in the longest back muscle. Among the essential amino acids, lysine has the highest content, while methionine has the lowest. Among the non-essential amino acids, glutamic acid predominated, with serine being the least abundant. The amino acid composition of beef also depends on different parts of the carcass. A comparison of different parts of beef within the same breed **[47]** showed that the most common amino acids in their composition (in descending order) were glutamic acid, aspartic acid, lysine, leucine, cysteine, arginine, glycine, and phenylalanine. However, histidine and methionine were detected in much smaller amounts and their quantitative values were almost the same regardless of the part of the carcass.

Other scientists confirmed our conclusion in their studies **[16]**. A similar issue is studied in the paper Hollo et al. **[17]**. When studying the amino acid composition of beef, Christensen et al. **[33]** also proved the breed dependence of the content of certain amino acids in black cattle concerning Hanwoo and Wagyu breeds.

As part of the solution to the problem of providing the population with high-quality food products that meet medical standards and developing the concept of managing the production of safe products for consumption, further research should be conducted in the area of developing a general methodology for assessing the fatty acid composition of beef, taking into account the breed and age characteristics of animals, milk quality depending on the technological conditions of productive animals. A key component in solving this problem is the technology of keeping and feeding animals, feed quality **[48]**, **[49]**, **[50]** as well as veterinary welfare **[51]**, **[52]**, **[53]**. The availability of systematic research on the combined assessment of the biological value of proteins and the lipid composition of beef will allow the development of technologies for its intensive production based on individual breeds, thereby improving product quality while reducing economic energy and feed costs.

CONCLUSION

The balance of beef protein in terms of amino acid composition is crucial for predicting the protein completeness of new products. The obtained breed and age characteristics of the formation of the biological value of beef protein made it possible to determine its compliance with the human body's physiological needs, which is a prospect for expanding the range of food products with a balanced amino acid composition. The age of bulls is a limiting factor in the formation of the biological value of beef. With an increase in age from 3 to 21 months of intensively reared bulls of dairy and combined breeds, there is a gradual increase in the biological value of proteins, which is confirmed by the values of the amino acid index, but its formation is not straightforward. The presence of the first peak increase in the average values of the amino acid index in beef from bulls at 12 months of age (089%), followed by a steady decrease to 15 months (0.68%) and a repeated increase in the values in animals at 21 months (0.83%) was noted. Beef obtained from bulls of 21 months of age was characterised by a fairly high biological value compared to 3 months of age due to an increase in the rates of amino acid values for methionine by 0.16%, isoleucine – by 0. 16%, histidine – by 0.42%, arginine – by 0.18%, threonine – by 0.20%, valine – by 0.05%, leucine – by 0.08%, phenylalanine – by 0.11% and lysine – by 0.15%. This, in turn, ensures its potential ability to be sold in the retail network without restrictions on higher grades. The bull breed also had a significant influence on the differences in the values of the scores of individual amino acids in carcass flesh during ontogeny. However, the most significant differences between the breeds studied were manifested within a single age period. Since there is still no clear justification for the optimal slaughter age of young cattle of different productivity directions bred in Ukrainian farms, the results suggest that the rational age for slaughtering bulls of the studied breeds is 18-21 months. In comparison, for veal production it is 12 months. However, the obtained data should first be consistent with the regulated norm of the live weight of animals for the corresponding age period.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

The experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On the Protection of Animals from Cruelty") as amended as of 04.08.2017, and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985) (Simmonds, 2017) **[54]**. The research program was reviewed and approved by the Bioethics Committee of the Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine in accordance with the current procedure, No. 1 (15.01.2020).

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