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Amino Acid Content of Fallow Deer (Dama dama) Muscles from Organic and Conventional Feeding Grounds

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Amino Acid Content of Fallow Deer (*Dama dama*) Muscles from Organic and Conventional Feeding Grounds

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Abstract: The study aimed to determine the protein content and amino acid profile of the longissimus lumborum and the semiomembranosus muscles of fallow deer originating from an organic feeding ground (OFG) and a conventional feeding ground (CFG). The amino acid content was determined using the ion-exchange chromatography method in accordance with AOAC. The present study provides the first published data on the nutritional value of fallow deer meat proteins. An analysis of the results revealed significant differences between the essential (EAA) and non-essential amino acid (NEAA) contents, depending on the feeding ground. A higher nutritional value was noted for the OFG fallow deer muscles, as indicated by the higher EAA and NEAA amino acid contents, a higher EAA proportion, a higher chemical score (CS) value, and the calculated expected protein efficiency ratio (PER) and biological value (BV). A significant effect of the muscle type on the histidine content and the feeding ground x muscle interaction for glycine was noted. All fallow deer muscle samples exhibited a complete profile of essential amino acids and a high-quality protein profile. The data presented in this overview show that the nutritional value of fallow deer meat represents an excellent source of proteins for humans.

Keywords: meat; protein; nutritional value; biological value; breeding systems; game; venison

1. Introduction

Modern consumers are increasingly interested in natural products. This is linked to a growing awareness, particularly among the wealthier segments of society in the EU countries, of the relationship between diet and the incidence of chronic non-communicable diseases, including cardiovascular diseases, diabetes mellitus, obesity, mental health problems, neurological disorders and cancers [1]. In this era of very rapid civilisational development and concern for health, consumers are increasingly paying attention to the quality, nutritional value and origin of food products [2–6]. The comfort of animals and high rearing standards have a significant effect on obtaining the highest-quality products. There is, therefore, a growing interest in organic and traditionally manufactured products with exceptional sensory and health-promoting values [7]. Studies [8,9] show that organic meat is a food product that can reduce the risk of developing cancer. Some studies provide evidence of differences in the chemical composition between organic and conventional food [10]. From both an evolutionary and historical perspective, meat has been valued by human communities as a highly nutritious food [11]. Conscious consumers demand that the meat they buy is sourced from ethical production systems [12]. Recent years have observed an increase in the demand for game, as global consumption exceeded 2 million tonnes in 2023 [13–15]. Hence, various cervid species are bred in many countries [1,16]. Poland is among the leading game producers in Europe, and a rapid development of cervid breeding took place after Poland's accession to the EU. Both the growth and development of cervid breeding are justified in terms of supplying the population with high-quality food. It is also important for economic growth and sustainable environmental development [17]. Cervid production systems make use of poor land that is unsuitable for agricultural use. According to the data [18], the fallow deer population in Poland in 2023 amounted to 35,000 individuals and was 24% higher compared to 2015. In 2023, Game Breeding Centres kept 8,500 fallow deer, of which 2,500 animals were culled. The animals are kept in their natural habitat and use natural pastures, with their diet being predominated by grasses (up to 60%), legumes and herbs (40-50%), and tree leaves and shoots, acorns and shrubs accounting for 10-30% [19,20].

Low-quality dietary protein potentially leads to protein intake being significantly lower than the recommended daily allowance (RDA). A recent study by Moughan [21] showed that more than 100 countries worldwide are struggling with inadequate protein supply (after taking into account bioavailability) for their populations, even though analyses showed a protein surplus in almost all the countries. From a nutritional point of view, protein is the most valuable component of meat. As reported by Smith [22], the low amount of animal protein consumed per person per day worldwide, which ranges from 7 to 77 g, is alarming. In addition, the proportion of elderly people with increased protein requirements in high-income countries has been on the increase [22]. In countries such as the UK, the Netherlands, Japan and Italy, 20-35% of the elderly consume less protein than the recommended daily allowance [22,23]. A similar situation is found in North America, where approximately 40% of the elderly consume less protein than recommended [22,24]. It is often overlooked that the protein RDA of 0.83 g/kg of body weight per day recommended by the FAO/WHO [25] refers to high-quality protein. In 2022, Vienna, Austria, hosted a meeting of FAO experts who proposed an accurate measurement and assessment of the quality of protein in human foods and diets. It is, therefore, of utmost importance that people of all ages and physiological conditions be provided with an adequate amount of dietary protein to meet their requirements for essential amino acids [23]. Not only are amino acids important building blocks of proteins, but they also play a crucial role in numerous processes that take place in the body, including the production of hormones, enzymes, neurotransmitters and bodily fluids [26]. They also serve a significant role in the regulation of mood and mental health through their involvement in the synthesis of hormones and neurotransmitters [27]. Their beneficial effects are experienced by people leading active lifestyles [28]. Consequently, ensuring an adequate supply of dietary protein at the individual and population levels is crucial to considering global food and nutrition security [21].

To maintain good health, it is important to consume low-fat animal protein and meat [29]. Cervid farming provides an opportunity for producers to satisfy consumer demand for lean meat. Several studies have been conducted on the basic physical and chemical characteristics, fatty acid and mineral contents, and the sensory characteristics of cervid meat. The differences in meat composition and quality between animals are mainly due to the species, age and sex [30,31]. It has been demonstrated that the differences in the environment and feed also affect the nutritional value of meat [7,31,32]. Publications on the quality of fallow deer meat are scarce [4,7,30,33,34], and the aspects of meat protein quality have not been studied. Only a comprehensive analysis of protein allows an assessment of its nutritional value to be carried out correctly [35]. Therefore, the main objective of the present study was to determine the amino acid content and the biological value of proteins in the LL and SM muscles of two selected groups of fallow deer grazed on organic and conventional feeding grounds.

2. Materials and Methods

2.1. Animals and Their Habitat

The fallow deer (*Dama dama*) originated from the southeastern region of Poland. A detailed description of the organic and conventional farms is provided in the previous paper [7]. It should be noted that 191 plant species were identified on the organic feeding ground (OFG), with dicotyledonous greens (44.5%) and legumes (22.5%) dominating the undergrowth. On the conventional feeding ground (CFG), 72 species were recorded, with the highest proportion of dicotyledonous greens (43%) and grasses (22%). The animals were culled with the permission of veterinary services during the autumn season and divided into groups by age and sex. The carcasses were then transported to a processing plant, where a veterinary examination was carried out. In the plant, after 24-hour cooling (at 4°C), 12 OFG fallow deer carcasses and 12 CFG fallow deer carcasses

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were randomly selected. Both groups comprised six does and six bucks in an equal sex ratio, and aged approximately 24 months. Samples of the longissimus lumborum (LL) and semimembranosus (SM) muscles were collected from each right fallow deer carcass, then placed in sterile, hermetically sealed Ziploc bags under refrigerated conditions (4oC) and transported to a laboratory where chemical analyses were carried out.

2.2. Chemical Analysis

The protein content was assessed by the Kjeldahl method [36]. Briefly, 0.5 g of the sample was digested using sulfuric acid, and the ammonia distilled into boric acid. The borate anions were titrated with hydrochloric acid and the crude protein content expressed by multiplying the analyzed total nitrogen content by 6.25. The analysis of amino acids (except cystine, methionine and tryptophan) in meat was carried out using ion-exchange column chromatography after etching the sample with 6N hydrochloric acid at 110°C for 24 hours in accordance with the AOAC method [36]. After evaporation of the hydrolysate, the amino acids were dissolved in citrate buffer (pH 2.2). The solution prepared in this way was collected for chromatographic analysis. Cysteine and methionine of the protein were oxidised with performic acid to cysteic acid and methionine sulphonate. The tryptophan content was determined in a sample of the protein hydrolysed in a barium hydroxide solution at 110°C for 18 h. Barium ions in the hydrolysate were precipitated with sulphuric acid. The precipitate was centrifuged, and the solution was transferred to a volumetric flask. The precipitate was rinsed with citrate buffer (pH = 2.2), with the solution being transferred each time to a volumetric flask [37]. The solution prepared in this way was collected for chromatographic analysis. The chromatographic analysis was performed in an AAA 400 amino acid analyser manufactured by INGOS (Czech Republic) with an ion-exchange column and a UV-VIS detector. The results were expressed in mg/g of the muscle tissue.

2.3. Assessment of Protein Nutritional Value

The chemical measure of protein quality CS (chemical score) was determined as a ratio of the exogenous amino acid content of the test protein (g/100 g of digestible protein) to the content of the same amino acid in the standard protein (g/100 g of protein), using three amino acid standards for adult humans [38–40].

The essential amino acid index (EAAI) was calculated as the geometric mean of all exogenous amino acids to the content of these amino acids in a particular standard.

The protein quality was estimated using empirical models originally developed by Alsmeyer et al. [41] and Lee et al. [42] for predicting the PER value. The present study used the following equations:

$$PER1 = -0.468 + 0.454 \times Leu - 0.104 \times Tyr$$

$$PER2 = -1.816 + 0.435 \times Met + 0.780 \times Leu + 0.211 \times His - 0.944 \times Tyr$$

$$PER3 = 0.08084 \times (X7) - 0.1094$$

where X7 = Thr + Val + Met + Ile + Leu + Phe + Lys

$$PER4 = 0.0063 \times (Thr + Val + Met + Ile + Leu + Phe + Lys + Arg + Tyr) - 0.1539$$

In order to calculate the biological value (BV), the following formula [43] was used:

$$BV = 1.09\ (EAAI) - 11.7.$$

2.4. Statistical Analysis

Statistical analysis was conducted using Statistica software (version 13.3, TIBCO Software Inc., Palo Alto, Kalifornia, USA). The normality was assessed using the Kolmogorov-Smirnov test. The effects of factors (feeding ground and muscle) on the analysed traits were evaluated using analysis of variance with interaction according to the model:

$$Y_{ij} = \mu + F_i + M_j + (FG \times M) + e_{ij}$$

where:

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μ – mean value, Fi – fixed effect of the feeding ground, Mj – fixed effect of the muscle, (FG x M) – interaction between factors (feeding ground, muscle), eij – random error.

Tukey's test was applied for the multiple comparisons among means within each muscle means of particular ground system (FG) were compared using t-test for independence groups. P value lower than 0.05 was considered as statistically significant.

3. Results

The results concerning the protein content of the LL muscle of fallow deer from the two feeding grounds are provided in Table 1. These values for the LL muscles of both OFG and CFG fallow deer were very similar, and no statistically significant difference was noted for the protein content. A total of 18 amino acids (AA) were detected in the fallow deer muscles. The effect of the feeding ground on the levels of all EAAs, with the exception of Trp, and on the TAA (total amino acids), acidic AA (AcAA) and aromatic AA (ArAA) contents was noted. Similarly, the feeding ground differentiated the NEAA content (with the exception of Cys). The His content was determined by the feeding ground and muscle type. The Gly content was affected by the feeding ground and the feeding ground x muscle interaction. The LL muscle of the organic feeding ground (OFG) fallow deer exhibited significantly higher Met, Thr and Phe contents compared to the LL muscle of the conventional feeding ground (CFG) fallow deer. Significantly higher sweet AAs contents (i.e. Ala, Gly, Pro, Ser and Thr) were noted for the LL muscle of the OFG fallow deer. In the muscles of the OFG fallow deer, significantly higher contents of all NEAAs (Arg, Ser, Asp, Glu, Pro, Gly, Ala and Tyr) were noted compared to the LL muscle of the CFG fallow deer (with the exception of Cys). The feeding ground type had a significant effect on the sum of all AAs (TAAs). Significantly higher TAA levels ($P \leq 0.009$) were noted for the LL muscle of the OFG fallow deer compared to the CFG fallow deer, which resulted from the higher proportion of EAAs and NEAAs. The muscle type and the feeding ground x muscle interaction had an impact on %EAA, %NEAA, EAA/NEAA and EAA/TAA. Higher and more favourable EAA/NEAA and EAA/TAA ratios were found in the LL muscle of the OFG fallow deer compared to the LL muscle of the CFG fallow deer.

The EAA content of the OFG fallow deer SM muscle, significantly higher than that in the CFG fallow deer muscle, was noted due to the higher proportion of the following AAs: Lys, Leu, His, Phe and Thr. Lis and Leu were the most abundant EAAs found in the SM muscle, similar to the LL muscle, whereas the lowest level was noted for Met. As for the NEAAs, a significant difference was noted for Glu and Tyr in the SM muscles of the OFG fallow deer. In the NEAA group, the highest amounts of glutamic acid were detected, followed by Asp and Arg, whereas the lowest value was noted for Cys. The SM muscle of the OFG fallow deer exhibited a significantly higher percentage of ArAA (Phe, Tyr and Trp).

Table 1. The protein (%) and essential and non-essential amino acid (mg/g) contents of the muscles of fallow deer from the two feeding grounds.

Specification	*Longissimus lumborum* (LL)					*Semimembranosus* (SM)					Impact of factors		
	OFG		CFG		P-value	OFG		CFG		P-value	FG	M	FG x M
	$\overline{X}$	SE	$\overline{X}$	SE		$\overline{X}$	SE	$\overline{X}$	SE		P-value		
Protein	23.17	0.57	23.19	0,66	0,987	23,88	0.74	23.90	0.61	0,984	0.970	0.216	0.986
Essential amino acids													
Threonine	8.58	0.29	7.94	0.17	0.021	8.80	0.33	8.09	0.26	0.050	0.004	0.412	0.879
Valine	9.08	0.32	8.46	0.24	0.151	9.65	0.37	8.88	0.27	0.083	0.024	0.106	0.798
Methionine	4.89	0.11	4.39	0.11	0.003	4.87	0.14	4.51	0.18	0.072	0.001	0.693	0.554
Isoleucine	7.69	0.28	7.20	0.12	0.061	7.99	0.32	7.36	0.29	0.083	0.012	0.282	0.748

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Leucine	14.78	0.31	13.94	0.24	0.064	15.44	0.55	13.99	0.33	0.036	0.005	0.364	0.443
Phenylalaine	7.31	0.20	6.82	0.15	0.032	7.65	0.30	6.98	0.11	0.047	0.004	0.206	0.654
Lysine	17.80	0.47	16.72	0.36	0.073	18.57	0.61	16.87	0.37	0.032	0.005	0.328	0.511
Tryptophan	7.74	0.10	7.92	0.20	0.368	7.98	0.16	8.16	0.25	0.487	0.269	0.145	0.996
Histidine	7.26	0.20	6.74	0.16	0.069	8.44	0.37	7.44	0.25	0.034	0.005	0.001	0.358
Non-essential AA													
Arginine	15.20	0.54	13.75	0.30	0.020	15.24	0.47	14.32	0.32	0.201	0.012	0.503	0.561
Serine	7.34	0.25	6.75	0.21	0.022	7.42	0.32	6.94	0.20	0.146	0.010	0.514	0.774
Aspartic acid	16.70	0.37	15.58	0.30	0.036	17.14	0.62	15.88	0.42	0.093	0.009	0.401	0.879
Glutamic acid	26.43	0.55	24.51	0.58	0.022	26.41	1.04	23.74	0.71	0.045	0.003	0.597	0.614
Proline	8.34	0.58	6.09	0.34	0.008	8.24	0.44	7.58	0.22	0.185	0.003	0.132	0.089
Glycine	9.12	0.41	7.70	0.21	0.008	8.58	0.31	8.73	0.31	0.731	0.046	0.456	0.020
Alanine	10.94	0.34	9.91	0.24	0.013	10.86	0.46	10.10	0.22	0.127	0.006	0.868	0.659
Cysteine	0.29	0.02	0.33	0.03	0.228	0.33	0.03	0.27	0.04	0.218	0.677	0.718	0.090
Tyrosine	6.83	0.26	6.25	0.13	0.009	7.08	0.27	6.42	0.22	0.033	0.001	0.238	0.827
Total amino acids	190.67	7.76	176.24	4.45	0.009	186.32	5.44	170.99	3.42	0.064	0.002	0.299	0.923
EAA	89.38	2.98	82.26	2.48	0.044	85.13	2.33	80.12	2.13	0.050	0.006	0.132	0.617
NEAA	101.29	3.91	93.97	2.23	0.004	101.18	3.47	90.87	2.55	0.082	0.051	0.535	0.562
% EAA	49.89	0.23	46.67	0.25	0.005	45.73	0.33	46.85	0.20	0.461	0.052	0.035	0.005
%NEAA	55.10	0.23	53.32	0.25	0.005	54.26	0.33	53.14	0.20	0.461	0.052	0.035	0.005
EAA:NEAA	0.98	0.007	0.87	0.020	0.005	0.84	0.015	0.88	0.020	0.466	0.057	0.038	0.006
EAA:TAA	0.56	0.002	0.46	0.007	0.005	0.45	0.010	0.46	0.007	0.461	0.052	0.035	0.005

OFG - organic feeding ground; CFG - conventional feeding ground; EAA - essential amino acids; NEAA - non-essential amino acids; TAA = total amino acids.

The LL muscle of the OFG fallow deer compared to the CFG, showed higher Met+Cys and Phe+Tyr contents (Table 2). The effect of the feeding ground on the AcAAs (Asp, Glu), ArAAs (Phe, Tyr, Trp), LNAAs (large neutral amino acids: Ile, Leu, Phe, Tyr, Val) and DAAs (delicious amino acids: Asp, Glu, Gly, Ala), was noted. In the LL muscle of the OFG fallow deer, AcAA, AaAA, LNAA and DAA levels were significantly higher than those in the muscle of the CFG fallow deer. The SM muscle of the OFG fallow deer exhibited significantly higher Phe+Tyr and LNAA contents than the CFG fallow deer muscle. The effect of the muscle on the Trp/LNAA and LNAA/Trp ratios was noted. A higher Trp/LNAA ratio value was noted for the CFG fallow deer muscles, whereas the muscles of the OFG fallow deer exhibited a higher LNAA/Trp ratio.

The data on the nutritional value of protein of the OFG and CFG fallow deer LL muscles is provided in Table 3. In all cases, the chemical score (CS) index exceeded 100%. Significantly higher CS index values were noted for Thr, His, Phe+Thr and Met+Cys in the LL muscle of the OFG fallow deer. The PER 3 and PER 4 index values were significantly higher for the LL muscles of the OFG fallow deer than for the CFG fallow deer muscles. The LL muscle of the fallow deer from the two feeding grounds did not differ significantly in terms of the protein biological value (BV). In the SM muscle of the OFG fallow deer, a CS index value being significantly higher for Lys, His and Leu as well as Phe+Tyr than for the CFG fallow deer muscle was noted. The SM muscle of the OFG fallow deer exhibited a significantly higher PER index value than that of the CFG fallow deer muscle. As for the biological value of protein, no significant differences were noted for the feeding grounds under study.

Table 2. Amino acid totals and percentages, and AA proportions in fallow deer muscles.

Specification	*Longissimus lumborum* (LL)					*Semimembranosus* (SM)					Impact of factors		
	OFG		CFG		P-value	OFG		CFG		P-value	FG	M	FG x M
	$\overline{X}$	SE	$\overline{X}$	SE		$\overline{X}$	SE	$\overline{X}$	SE		P-value		
Met+Cys	5.19	0.29	4.77	0.16	0.003	5.18	0.16	4.71	0.10	0.064	0.999	0.617	0.879
Phe+Tyr	14.73	0.57	13.39	0.33	0.016	14.14	0.34	13.06	0.26	0.039	0.998	0.228	0.989
AcAA	43.54	5.50	39.62	1.68	0.022	43.12	1.13	40.08	0.72	0.052	0.004	0.984	0.699
ArAA	22.70	0.16	21.55	0.61	0.080	21.88	0.34	20.98	0.35	0.153	0.031	0.138	0.787
%AcAA	22.81	0.24	22.46	0.26	0.431	23.17	0.22	23.44	0.30	0.242	0.051	0.206	0.654
%ArAA	11.93	0.11	12.23	0.13	0.009	11.77	0.15	12.28	0.11	0.066	0.050	0.138	0.511
BCAA	33.07	1.09	30.23	1.44	0.069	31.55	0.91	29.59	0.65	0.051	0.001	0.214	0.604
%BCAA	17.33	0.25	17.15	0.17	0.070	16.93	0.27	17.29	0.13	0.347	0.513	0.350	0.049
BCAA:ArAA	1.44	0.02	1.41	0.02	0.284	1.45	0.02	1.40	0.01	0.074	0.052	0.865	0.593
DAA	62.98	2.71	58.44	1.17	0.009	63.18	2.38	57.70	1.86	0.105	0.004	0.870	0.775
%DAA	33.00	0.26	33.14	0.30	0.666	33.92	0.39	33.74	0.35	0.714	0.942	0.007	0.570
DAA:TAA	0.33	0.009	0.33	0.009	0.666	0.33	0.010	0.33	0.010	0.714	0.943	0.007	0.570
LNAA	47.80	2.79	43.62	1,42	0.038	45.69	1.71	42.66	1.07	0.043	0.004	0.202	0.632
%LNAA	25.05	0.12	24.75	0.16	0.053	24.53	0.25	24.94	0.19	0.113	0.698	0.212	0.012
Trp:LNAA	0.16	0.01	0.19	0.01	0.010	0.15	0.01	0.18	0.01	0.004	0.000	0.921	0.754
LNAA:Trp	5.98	0.25	5.36	0.16	0.012	5.89	0.15	5.41	0.20	0.004	0.000	0.872	0.618

FFG - forest feeding ground; OFG organic feeding ground; CFG - conventional feeding ground; AcAA - acidic amino acids; ArAA – aromatic amino acids; BCAA - branched-chain amino acids; DAA - tasty amino acids, LNAA - large neutral amino acids.

Table 3. Indices of nutritional value of fallow deer muscle protein.

Specification	*Longissimus lumborum* (LL)					*Semimembranosus* (SM)					Impact of factors		
	OFG		CFG		P-value	OFG		CFG		P-value	FG	M	FG x M
	$\overline{X}$	SE	$\overline{X}$	SE		$\overline{X}$	SE	$\overline{X}$	SE		P-value		
CS 1 Isoleucine FAO/WHO 1991	285.38	10.33	262.91	6.98	0.061	274.64	8.44	257.08	6.65	0.083	0.012	0.282	0.748
CS 2 Isoleucine USA 2002	320.14	11.88	294.93	6.54	0.061	308.09	8.41	288.39	6.92	0.083	0.012	0.282	0.748
CS 3 Isoleucine FAO/WHO 2013	265.65	10.75	244.73	5.53	0.061	255.65	7.75	239.30	5.36	0.083	0.012	0.282	0.748
CS 1 Leucine FAO/WHO 1991	234.16	9.69	212.25	5.38	0.064	224.16	5.67	211.40	4.23	0.036	0.005	0.364	0.443
CS 2 Leucine USA 2002	280.44	14.36	254.20	6.02	0.064	268.47	7.16	253.19	15.05	0.036	0.005	0.364	0.443
CS 3 Leucine FAO/WHO 2013	291.46	15.71	264.19	6.88	0.064	279.01	7.99	263.13	15.72	0.036	0.005	0.364	0.443
CS 1 Lysine FAO/WHO 1991	320.68	17.73	291.26	6.84	0.073	307.27	8.60	288.61	6.62	0032	0.005	0.328	0.511
CS 2 Lysine USA 2002	363.91	12.81	330.52	8.06	0.073	348.69	9.18	327.52	7.54	0.032	0.005	0.328	0.511
CS 3 Lysine FAO/WHO 2013	413.12	28.60	375.22	10.72	0.073	395.85	10.27	371.81	9.86	0.032	0.005	0.328	0.511

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CS 1 Methionine+Cysteine FAO/WHO 1991	208.30	13.75	191.30	6.68	0.003	207.63	4.48	189.00	3.38	0.064	0.001	0.775	0.875
CS 2 Methionine+Cysteine USA 2002	208.30	13.75	191.30	6.68	0.003	207.63	4.48	189.00	3.38	0.064	0.001	0.775	0.875
CS 3 Methionine+Cysteine FAO/WHO 2013	235.47	16.85	216.25	7.12	0.003	234.71	5.37	213.65	4.99	0.064	0.001	0.775	0.875
CS 1 Phenylolanine+Tyrosine FAO/WHO 1991	234.26	19.22	213.06	5.25	0.016	224.93	6.27	207.75	4.80	0.039	0.002	0.217	0.733
CS 2 Phenylolanine+Tyrosine USA 2002	313.15	19.06	284.81	7.72	0.016	300.68	7.43	277.70	5.45	0.039	0.002	0.217	0.733
CS 3 Phenylolanine+Tyrosine FAO/WHO 2013	386.83	18.25	351.82	8.83	0.016	371.43	9.17	343.05	7.79	0.039	0.002	0.217	0.733
CS 1 Threonine FAO/WHO 1991	259.38	10.62	238.45	6.51	0.021	253.02	6.40	234.08	5.86	0.058	0.004	0.412	0.879
CS 2 Threonine USA 2002	325.38	18.42	299.12	8.47	0.021	317.40	7.59	293.63	6.15	0.058	0.004	0.412	0.879
CS 3 Threonine FAO/WHO 2013	381.87	15.09	351.05	8.72	0.021	372.50	10.03	344.61	7.82	0.058	0.004	0.412	0.879
CS 1 Tryptophan FAO/WHO 1991	722.60	21.25	738.82	28.07	0.368	701.23	5.81	717.31	16.34	0.487	0.269	0.145	0.996
CS 2 Tryptophan USA 2002	1133.16	24.70	1158.61	36.74	0.368	1099.66	12.05	1124.88	25.22	0.487	0.269	0.145	0.996
CS 3 Tryptophan FAO/WHO 2013	1312.08	24.91	1341.55	36.60	0.368	1273.30	18.69	1302.49	28.68	0.487	0.269	0.145	0.996
CS 1 Valine FAO/WHO 1991	276.68	16.05	254.53	7.13	0.151	260.41	11.26	242.66	8.00	0.083	0.024	0.106	0.798
CS 2 Valine USA 2002	301.58	19.30	277.44	8.12	0.151	283.85	14.07	264.50	9.43	0.083	0.024	0.106	0.798
CS 3 Valine FAO/WHO 2013	321.26	11.86	295.55	8.70	0.151	302.37	16.30	281.76	9.35	0.083	0.024	0.106	0.798
CS 3 Histidine FAO/WHO 1991	484.00	10.47	449.33	9.62	0.028	562.66	12.48	496.00	13.56	0.030	0.001	0.775	0.875
PER 1	5.80	0.28	5.21	0.20	0.090	5.53	0.29	5.20	0.18	0.039	0.007	0.390	0.414
PER 2	7.43	0.30	6.56	0.20	0.161	6.92	0.24	6.48	0.29	0.050	0.016	0.259	0.405
PER 3	5.78	0.28	5.28	0.12	0.033	5.56	0.14	5.18	0.17	0.039	0.003	0.258	0.650
PER 4	6.40	0.28	5.84	0.17	0.025	6.13	0.11	5.67	0.10	0.046	0.003	0.184	0.751
% EAAI 1	181.70	5.56	172.74	3.96	0.088	176.04	3.17	169.20	2.54	0.128	0.026	0.185	0.757
% EAAI 2	226.65	5.49	216.87	5.01	0.126	219.64	4.08	212.31	3.42	0.169	0.045	0.170	0.768

% EAAI 3	249.34	6.07	238.89	5.58	0.133	241.69	4.09	233.86	3.44	0.178	0.049	0.168	0.774
Biological value 1	180.18	3.09	172.73	2.31	0.088	186.36	4.97	176.59	4.04	0.128	0.051	0.169	0.767
Biological value 2	227.71	13.18	219.73	11.37	0.126	235.35	20.16	224.69	16.36	0.169	0.051	0.169	0.767
Biological value 3	251.75	14.27	243.21	12.47	0.133	260.08	21.88	248.69	18.08	0.178	0.051	0.169	0.767

OFG - organic feeding ground; CFG - conventional feeding ground; CS - chemical score ; PER – protein efficiency ratio; EAAI - essential amino acid index.

4. Discussion

From a human nutrition point of view, protein is the most valuable component of meat. The nutritional value of meat is primarily determined by the amino acid content and composition of protein. Protein is a key nutritional substance that supports the growth and development of the human body [11,22,44]. Proteins ensure the maintenance of a correct water balance in the body by being involved in regulating fluid content, thus supporting immunity [45]. In addition, thanks to their buffering properties, proteins are involved in the regulation of the acid-base balance [44]. Providing protein in the diet is, therefore, essential to maintaining the proper functioning of the body. Proteins of animal origin are often preferred to plant proteins, as they are believed to better support the synthesis of muscle proteins [28]. Meat proteins are highly digestible and easily absorbed [46]. A recommended dietary allowance (RDA) based on the optimal requirement to maintain nitrogen balance in the body [25] is 0.83 g/kg of body weight per day for adults of all ages, with the exception of pregnant and breastfeeding women and the elderly. More recent studies using stable isotope tracers suggest that the daily protein requirement in adults may be higher and amount to approximately 1.1-1.2 g/kg of body weight per day [47]. An increased requirement for protein is also noted in chronically ill patients [11].

The amount of protein contained in 100 g of fresh fallow deer meat, noted in the present study, provides one-third of the daily minimum protein requirement for adults. The protein content of fallow deer meat (23.17-23.90 mg/g) was similar to the protein content noted by Bureš et al. [32] for farmed fallow deer and by Wach et al. [4] for wild fallow deer. Lower values for fallow deer have been reported by Piaskowska et al. [48], Cawthorn et al. [30] and Ivanović et al. [34]. It is also noteworthy that the protein content of fallow deer meat is usually higher than that of livestock meat. This thesis is supported by numerous publications on the chemical composition of pork, beef and lamb, in which the protein content ranged from 18.7-20% for the longissimus dorsi in pigs of the native breed [49], from 22-22.6% for cattle [50], and from 20-21% for rapid-growing lambs [51].

An accurate assessment of the nutritional quality of dietary proteins is of fundamental importance, as the human body requires 20 EAAs in order to maintain the normal course of many vital processes. Eight of these AAs cannot be synthesised in the human body, which means that they must be present in the human diet [52]. Arginine and histidine are considered essential for infants [53], whereas the remaining AAs are considered to be non-essential. From a human perspective, the so-called essential amino acids are the most important. These include phenylalanine, leucine, isoleucine, lysine, methionine, threonine, tryptophan and valine. An assessment of the nutritional value of protein involves both chemical methods (AA analysis) and biological methods (the degree of protein utilisation by the living organism) [35], with these methods complementing each other. The view is taken that only a comprehensive assessment while applying chemical methods and a selected biological method, allows valid results to be obtained. According to the FAO recommendations, amino acids should be considered individual nutritive substances [40]. Great attention is paid to the action of exogenous, branched-chain amino acids (BCAA) [54]. Leucine, isoleucine and valine make up the BCAA group. The BCAA content in the present study was similar to the results obtained by Kasprzyk [55] for deer from the organic and conventional feeding grounds. The meat of the studied fallow deer exhibited a BCAA content twice as high as that of meat of rapidly growing lambs fed different bulky feed types [51]. BCAAs contribute to an improvement in mood due to the synthesis of serotonin [56]. They serve an important role in the proper functioning of the nervous system, as the neurotransmitter glutamate is formed during the metabolic conversions of these compounds [57]. In addition, BCAAs are essential for the proper functioning of immune cells [26]. Studies [57,58] suggest that BCAAs significantly stimulate muscle mass gain, contribute to muscle regeneration, reduce the amount of adipose tissue, and neutralise the effects of physical fatigue. Branched-chain amino acids are a common ingredient of dietary supplements, as they contribute to the stimulation of the synthesis of proteins that are the building material of the muscles [56]. They are an important dietary component in cases of obesity, diabetes mellitus, cardiovascular diseases, metabolic syndrome and infertility in humans [44,56]. BCAAs stimulate insulin secretion [59]. Leucine is an AA belonging to the BCAA group. The daily leucine requirement for an adult is 2.7 g and can be satisfied by consuming 150 g of fallow deer meat. Since the diets of most people in the world are rich in cereals, which contain relatively little lysine, the consumption of 100 g of fallow deer meat can fully satisfy the lysine requirement. Lysine is an AA that is essential for ensuring normal growth and development of the bones in children [52,60], is involved in hormone synthesis and antibody production, and is a crucial component required for collagen production [61].

The significant presence of functional AAs (Ala, Arg, Asp, Glu, Gly, Leu, Met, Phe, Pro, Thr and Tyr) in the OFG fallow deer meat is extremely important from a health point of view because by controlling certain metabolic pathways they modulate the immune system and help treat certain metabolic disorders [25]. Meat proteins may be more effective in preventing sarcopenia than soybean [62]. The LL muscle of the OFG fallow deer is abundant in aspartic and glutamic acids. Asp belongs to the NEAAs and, at the same time, is an agent that limits the growth of certain neoplasms [63,64]. Arginine therapy may exert neuroprotective effects after ischaemic brain injury [65]. The functions of Glu in the human body are diverse. It is involved in maintaining the acid-base balance, in water transport and nitrogen metabolism. It also supports the immune functions of the body, improves gastrointestinal function, and enhances concentration and memory [25]. Clinical studies demonstrated that Glu supplementation is recommended in the early stages of Covid-19 infection, as it reduces the severity of symptoms and strengthens the immune system [66,67]. Glutamine is particularly recommended in states of increased metabolic stress, e.g. in the case of neoplastic disease, as its synthesis is insufficient, and it consequently must be supplied with food [54]. Therefore, the wider introduction of organic fallow deer meat into the diet may significantly reduce the incidence of lifestyle diseases [68].

The LL muscle of the OFG fallow deer exhibited higher AcAA and ArAA contents than the LL muscle of the CFG fallow deer. Similarly, a study by Kasprzyk [55] noted a higher ArAA content of the muscles of fallow deer from the organic feeding ground. Aromatic and acidic amino acids can affect brain function. Tryptophan is used in the treatment of sleep disorders and depression [69]. Tryptophan is a precursor for serotonin and melatonin, whereas "tyrosine and phenylalanine are substrates for neurotransmitters: dopamine and noradrenaline, respectively" [70]. Sulphur-containing amino acids, e.g. cysteine and methionine, are essential for the synthesis of glutathione and taurine, increase the level of the body's antioxidant defence, and provide a defence against oxidative stress [54]. In the authors' own study, the best source of Met proved to be the OFG fallow deer muscles. According to a study by Singhal et al. [69], methionine supplementation proved to be effective in patients with multiple sclerosis. In the present study, the high DAA content (over 57 mg/g) is noteworthy. A lower content (38 mg/g) was observed by Li et al. [51] in sheep meat.

The author own study observed that fallow deer meat exhibited a high EAA content. At the same time, the amino acid composition of fallow deer muscles was found to be closely linked to the feeding environment and the fodder naturally available there. As indicated by Bureš et al. [32], the AA content of ruminant meat is determined by the profile of assimilable amino acids (AA) contained in the fodder, as a deficiency of a single AA may reduce the utilisation of other AAs. It has been demonstrated that the more EAAs, the higher the nutritional value of meat [71], which means that the OFG fallow deer muscles are more nutritious than the CFG fallow deer muscles, as a smaller portion of meat can better satisfy the human requirement for essential amino acids. Similar values for the EAA in deer were noted in the study by Kasprzyk [55]. Interestingly, the EAA content of the fallow deer under study (80-89 mg/g) is higher than that in livestock meats such as beef: 49.8 mg/g [72], lamb: 47.9 mg/g [72], (40-41 mg/g) [51] and pork: 50 mg/g [72], (37-41 mg/g) [73]. EAAs also play an important role in mitochondrial biogenesis and dynamic immune homeostasis [74].

In 2013, the FAO/WHO Expert Committee adopted a new standard protein composition [40] (Table 4). This composition is currently considered to be the most suitable for evaluating protein in the nutrition of all human groups. In the CFG fallow deer muscle meat, the Lis, Met, Cys and Val contents were similar to those of standard protein, whereas the remaining AAs were found at levels higher than those for the reference protein. Particularly favourable contents included Lys as well as Met and Cys, which are the most important AAs in terms of nutritional value [26]. In the fallow deer muscle tissue proteins, the EAA content (in g/100 g) was, on average, 53% higher than the AA level in reference protein proposed by FAO/WHO/UNU [40]. In addition, the total amount in the meat of the animals under study ranged from 41.12 to 43.10 [g/100 g protein], whereas in standard protein, this amount is approximately 27.7 [g/100 g]. The EAA content reported in beef jerky was only 38 g/100 g of protein [75], while in lamb meat it ranged from 35.6-37.8 g/100 g of protein [76]. In the authors' own study, the total amount of EAAs in fallow deer meat accounted for an average of 47.3% of all AAs, which was a value higher than that recommended by the WHO/FAO/UNU experts, which accounted for approximately 29% [25]. The authors' own study noted more than twice as many NEAAs than those in beef [75] and the meat of native Prestice Black-Pied pigs (40 mg/g) [73].

Table 4. Content of essential amino acids in the protein of red deer from the organic and conventional feeding ground [g/100 g protein].

Amino acids	**LL**		**SM**		**Standard protein* [g/100 g protein]**	**Standard protein* [mg/kg per day]**
	OFG	CFG	OFG	CFG		
Threonine	3.72	3.42	3.72	3.38	2.30	15
Valine	3.94	3.72	4.08	3.78	3.90	26
Methionine + Cysteine	5.5	2.22	5.58	2.15	2.20	15
Isoleucine	3.33	3.11	3.38	3.08	3.00	20
Leucine	6.40	6.01	6.54	5.85	5.90	39
Phenylalanine + Tyrosine	6.14	9.19	6.23	9.34	3.80	25
Lysine	7.72	7.21	7.85	7.06	4.50	30
Tryptophan	3.35	3.42	3.38	3.41	0.60	4
Histidine	3.15	2.91	3.56	3.11	1.50	10
Total essential amino acids	43.10	41.13	44.18	41.12	27.70	-

*According to FAO [40]; OFG - organic feeding ground; CFG - conventional feeding ground.

From a nutritional perspective, it is important to assess the EAAs-to-NEAAs ratio, as this is the basis for the classification of proteins into complete, semi-complete and incomplete proteins [54]. The EAA/TAA ratio was 56% (in the LL muscle) and 45% (in the MS muscle), which was in line with the ideal protein standard for humans (40%), as recommended by the FAO/WHO. The EAA/NEAA ratio in the two groups of fallow deer under study accounted for over 80%, which significantly exceeded the high-quality FAO/WHO standard proteins [40]. An EAA/NEAA ratio in goat meat, similar to that in the authors' own study, was reported by Brzostowski et al. [77] and Han [78]. Lower values were definitely noted for algae [79]. The muscles of the fallow deer under study exhibited the correct Trp/LNAA ratio, which may contribute to an improvement in mood and a reduction in stress [80].

The quality of dietary proteins is usually defined by the extent to which the constituent amino acids meet the amino acid needs of consumers [25]. The determination of the protein quality by the chemical method uses the term of so-called "limiting amino acid". It is an exogenous amino acid that is found in a particular protein in the lowest amount compared to standard protein. Taking into account the recent recommended amino acid scoring standards for adults, all the calculated average chemical results (CS) exceeded 100, which indicates the high quality of the studied fallow deer muscle protein. This is because the basis for the utilisation of protein supplied with food is its complete absorption in the gastrointestinal tract. A particularly high CS index value was noted for Lys, Thr, Phe and Trp. The CS value for Trp ranged from 1,099 to 1,312% and exceeded twice the FAO/WHO standard. In addition, the CS values for all EAAs calculated in the present study were similar to the values observed by Kasprzyk [55] in deer muscles and exceeded the values for the EAAs identified in the muscles of horses assessed by Lorenzo et al. [81].

Protein is of high quality when the EAAI value is higher than 90%, of moderate quality when this value is 70-89%, and of low quality when this value is lower than 70% [79]. High-quality protein indicates that the composition of essential amino acids is similar to the requirements of the human body [22]. In the present study, the EAAIs in the fallow deer muscles ranged from 169 to 249%. This means that the protein profile of fallow deer meat perfectly meets human requirements, and the calculated expected PRE and BV indices confirm the high nutritional value of meat, which is higher than that of dried beef [75] and casein and soybean [82]. In addition, the actual digestibility of meat protein amino acids in the ileum ranges from 90 to 100%, and the result for the digestible indispensable amino acids (DIAAs) under different cooking conditions ranges from 80 to 99% [83]. This further confirms that fallow deer meat is an excellent source of proteins and represents a very valuable component of the diet.

5. Conclusions

The composition of the amino acids consumed is one of the crucial aspects of human health. The study shows that there is a clear relationship between the fallow deer feeding ground and the amino acid composition of meat. The OFG fallow deer muscles are more nutritious than the CFG fallow deer muscles because of their higher EAA and NEAA contents and the biological value indices. Hence, a smaller portion of the OFG fallow deer meat can satisfy the requirement for essential amino acids. Irrespective of the differences demonstrated, the OFG and CFG fallow deer muscles under study were characterised by a good balance of nutritional amino acids and a very high proportion of nutritionally valuable essential AAs, which exceeds their content of the reference protein. The results support the greater use of fallow deer meat in the human diet to ensure good health. Fallow deer meat satisfies current consumer requirements thanks to sustainable production that ensures high animal welfare standards and contributes to the balance of amino acids in the diet, providing excellent potential for supplementing lower-quality proteins. Therefore, fallow deer meat is a good alternative to other red meats and can diversify the European meat market.

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Institutional Review Board Statement: This study was conducted under commercial farming practices with the appropriate farming measures and clinical activities which do not cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with Polish Policy for Animal Protection, which complies with the European Union Directive 2010/63/UE on the protection of research animals. Ethical review and approval were waived for this study, as all animal data collected was observational, and the assay was carried out as part of common zootechnical procedures. Muscle samples were collected for the analyses during the routine carcass cutting procedure at the meat processing plant.

Data Availability Statement: The data presented in this study are available in the article. Further inquiries can be directed to the corresponding author.

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