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AMINO ACID CONTENT AND BIOLOGICAL VALUE OF RABBIT MEAT PROTEINS, DEPENDING ON WEANING AGE

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Summary

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The amino acid content and biological value of rabbit meat proteins, expressed as essential amino acid index (EAAI) depending on weaning period were studied. For this purpose, 15 White New Zealand rabbits were divided into two groups: group A (n=7) – weaned at the age of 21 days, and group B (n=8) – weaned at the age of 35 days. The rabbits were slaughtered at the age of 90 days, in accordance with the requirements of humane treatment of animals. It was established that the weaning age of rabbits had an influence on the amino acid content and biological value of meat proteins. In rabbits weaned at the age of 35 days, the biological value of the proteins was higher due to the higher content of basic amino acids (lysine, histidine, arginine), leucines (leucine and isoleucine), and monoamino carboxylic acids (valine). Along with that, the biological value of m. Longissimus Lumborum meat proteins was higher than that of m. Semimembranosus meat. With regard to the amino acid content and biological value of proteins in meat, weaning of rabbits at the age of 35 days is recommended.

Key words: biological value of proteins, essential and non-essential amino acids, rabbit meat

INTRODUCTION

Rabbit meat is, to a great extent, compliant with the requirements for a complete protein diet and a reduction of fatty content of foods. According to Marinov *et al.* (2009), 100 g of rabbit meat contain 25 mg cholesterol, which is very close to its content in the meat of wild animals. Its energy content is low (160–170 kcal/100 g) compared to that of beef (195–380 kcal/100 g) and pork (260–330 kcal/100 g). Moreover, the high content of essential amino acids in rabbit meat emphasizes its dietetic properties.

Depending on the breeding technology, rabbits are weaned at different ages: with intensive rearing technologies on the 28th–30th day, with semi-intensive ones on the 28th–30th day, and with extensive rearing – at the age of 45 days (Marinov *et al.*, 2009). The recommended slaughtering age is 90 days (Grigorov, 2008). A number of authors have found that early weaned rabbits had a lower body mass at the end of fattening compared to rabbits weaned at the age of 32–35 days (Ferguson *et al.*, 1997; Trocino *et al.*, 2001; Gallois *et al.*, 2004; Vachkova *et al.*, 2010). On the other hand, according to Groen (1999), Gidenne *et al.*, (2004), and Zita *et al.* (2007), early weaning does not affect meat production, body mass during the fattening period, and meat quality.

Complete meat proteins contain sufficient amounts of essential amino acids, yet the quality of a protein, as a primary food component depends on its amino acid composition (Prior *et al.*, 1981; Taboada *et al.*, 1996; Wang & Parsons, 1998; Xiccato, 1999; Pla, 2004). That is why amino acids building the protein molecule are important for meat assimilation by the human body. Regarding the content of essential amino acids, rabbit meat is superior to the meat of some animal species (birds, fish). This has been proven by numerous studies on the amino acid content of the meat of goats (Ferreira, 2004), lambs (Löest *et al.*, 1997; Slavov, 2007), pigs (Cornet & Bousset, 1999; Okrouhla *et al.*, 2006), broiler chickens (Hamm, 1981; Ravindran *et al.*, 2005), and hens (Siddiqi *et al.*, 1994; Ruchii, 2007).

Interesting results were procured while studying the influence of various levels of muscle glycogen, lactate and amino acid metabolism during physical exercise in humans on the concentrations of amino acids in the leg muscles (Blomstrand & Saltin, 1999).

A review of literature revealed that existing studies on the amino acid content of rabbit meat are mostly related to the type of diet, feed ingredients, the influence of biologically active substances, probiotics, etc. (Colin & Ghezal-Triki, 2001; Matusevičius et al, 2006; Pla, 2008). Data on the effect of the rabbits' weaning age on the quality of the meat with regard to its amino acid content and biological value of proteins are scarce and inconsistent.

This study aimed to establish the amino acid content and the biological value of proteins in rabbit meat, depending on their weaning age.

MATERIALS AND METHODS

The experiment was performed at the vivarium of the Animal Physiology Department at the Faculty of Veterinary Medicine between February and April 2009.

Subject of this study were 15 White New Zealand rabbits, divided into two groups: group A (n=7) – weaned early, at the age of 21 days and group B (n=8) – weaned normally at the age of 35 days. Following weaning, the rabbits were kept in metal cages sized 80/60/40 cm, in a special premise. There were four rabbits per cage, yet they were regrouped to three per cage later as their age advanced, and after the age of 60 days only 2 rabbits per cage were left. The room was equipped with a heating appliance, natural and artificial light sources. The rabbits were given free access to food and water. Feeding was carried out using two types of commercial pelleted feed produced by Norex-Agro (Bulgaria): for weaned rabbits up to 50 days of age and growing rabbits of more than 50 days of age (Table 1). The cages and the room were cleansed regularly.

The temperature and humidity of the air in the room were detected simultaneously with a thermo hygrometre TZ 118. Concentrations of harmful gases (ammonia, hydrogen sulfide and sulfur dioxide) were measured using Hygitest indicator tubes.

At the age of 90 days, after being weighed, the rabbits were slaughtered under laboratory conditions in accordance with the prescriptions of the Commission for Humane Treatment of Animals at the Faculty of Veterinary Medicine.

Samples were obtain from m. Longissimus Lumborum (LL) and m. Semimembranosus (SM) of each carcass, which were chilled and kept for 24 h at 2 °C.

Table 1. Composition of commercial rabbit feeds

	For weaned rabbits	For growing rabbits
Ingredients, %		
Barley	5.000	5.000
Calcium carbonate	0.200	0.500
Sodium chloride	0.400	0.400
Alfalfa hay	34.000	28.000
Oats	30.870	31.875
Wheat brans	20.000	20.000
Dicalcium phosphate	0.300	0.300
DL-methionine 99%	0.100	
Vitamin and minerals premix for rabbits (Roche)	0.400	0.300
Sunflower meal	5.000	13.000
Soybean meal	3.000	
Mycofix +	0.100	
Pellet binder Wafolin-S	0.500	0.500
L-lysine 78%	0.080	0.100
Cycostat 66G	0.050	
Thyme	5.000	0.025
Chemical analysis, g/kg		
Dry matter	888.688	889.810
Crude protein	161.843	166.308
Crude fat	34.630	34.029
Crude fibre	151.643	153.979
Ash	69.080	52.013

The analysis of amino acids (essential and non-essential) in the meat was performed using ion-exchange column chromatography after acid digestion of the sample with 6N hydrochloric acid at 110° C for 24 h. The residue was dissolved in a buffer with pH 2.2. Sulfur-containing amino acids (methionine and cysteine) were identified after oxidation of the sample with hydrogen peroxide and performic acid. The separation of the different amino acids was performed on an amino acid analyser T 339 M (Mikrotechna, Praha), and their amount was calculated on the basis of elution and standard solution volumes.

The biological value of protein, expressed as essential amino acid index (EAAI), was assessed using the method of Oser (1951).

The results were processed statistically by ANOVA and the *post hoc* LSD test (Statistica v.6.1., Stat Soft Inc. 2002).

RESULTS

The data showed that the content of amino acids in the analysed muscles was statistically significantly different ($P<0.05$) with respect to weaning age. The essential amino acid index (EAAI) was 0.830± 0.010 for early weaned (21^{st} day) up to 0.837±0.020 for normally weaned (35^{th} day) rabbits.

The EAAI of SM was 0.826±0.010 for early weaned rabbits and 0.829±0.020 for

Table 2. Mean content of amino acid groups and (g/100 g protein) according to their chemical structure and essential amino acid index (EAAI) in m. Semimembranosus (SM) and m. Longissimus Lumborum (LL) of rabbits weaned at the age of 21 days (group A) and 35 days (group B). Data are presented as mean ±SD.

Amino acid groups	Group A			Group B		
	SM (n=7)	LL (n=7)	SM+LL (n=14)	SM (n=8)	LL (n=8)	SM+LL (n=16)
Σ monoamino carboxylic	16.12±0.40	16.06±0.17	16.09±0.29	17.02±0.51	17.27±0.54	17.14±0.52
Glycine	4.86±0.30	4.68±0.12	4.77±0.23	5.15±0.41	4.96±0.23	5.06±0.21
Alanine	5.91±0.12	5.92±0.05	5.92±0.09	6.07±0.14	6.05±0.26	6.06±0.11
Valine	5.35±0.13	5.46±0.20	5.40±0.17	5.80±0.11	6.26±0.31	6.03±0.20
Σ monoamino dicarboxylic	28.04±0.34	27.99±0.28	28.01±0.30	28.31±0.29	27.65±1.38	27.98±1.02
Aspartic acid	9.29±0.05	9.30±0.11	9.30±0.08	9.68±0.11	9.67±0.28	9.68±0.15
Glutamic acid	18.75±0.32	18.69±0.32	18.72±0.31	18.63±0.22	17.98±1.18	18.30±0.60
Σ ß-hydroxyamino carboxylic	7.43±0.13	7.29±0.09	7.36±0.13	4.77±0.31	5.08±0.31	4.92±0.34
Serine	3.19±0.10	3.07±0.06	3.13±0.10	1.55±0.17	1.75±0.18	1.65±0.34
Threonine	4.24±0.05	4.22±0.06	4.23±0.05	3.21±0.14	3.33±0.15	3.27±0.12
Σ basic	19.16±0.16	19.32±0.36	19.24±0.29	22.05±0.42	20.57±0.54	21.31±0.90
Lysine	9.21±0.10	9.27±0.08	9.24±0.09	9.78±0.19	9.97±0.18	9.88±0.12
Histidine	3.99±0.20	4.03±0.18	4.01±0.19	5.25±0.26	4.37±0.42	4.81±0.27
Arginine	5.96±0.08	6.02±0.24	5.99±0.18	7.02±0.21	6.23±0.16	6.63±0.10
Σ sulfur-containing	3.61±0.18	3.66±0.18	3.63±0.18	3.03±0.20	3.16±0.19	3.10±0.20
Methionine	2.46±0.06	2.46±0.12	2.46±0.09	1.98±0.08	2.02±0.16	2.00±0.09
Cysteine	1.15±0.19	1.20±015	1.17±0.16	1.05±0.17	1.13±0.14	1.09±0.09
Σ ring-containing	12.30±0.28	12.23±0.28	12.26±0.27	11.20±0.37	11.83±0.29	11.52±0.46
Phenylalanine	3.98±0.12	4.05±0.16	4.01±0.14	3.62±0.06	4.35±0.19	3.99±0.09
Tyrosine	3.24±0.09	3.21±0.07	3.22±0.08	2.54±0.15	2.75±0.21	2.65±0.12
Proline	5.08±0.29	4.97±0.20	5.03±0.24	5.04±0.33	4.73±0.45	4.89±0.30
Σ leucines	13.34±0.36	13.46±0.31	13.40±0.33	13.62±0.20	14.44±0.37	14.03±0.51
Leucine	8.44±0.26	8.50±0.24	8.47±0.24	8.72±0.14	9.15±0.22	8.93±0.15
Isoleucine	4.90±0.10	4.97±0.08	4.93±0.09	4.90±0.07	5.30±0.15	5.10±0.09
EAAI*	0.826±0.01	0.833±0.01	0.830±0.01	0.829±0.01	0.844±0.02	0.837±0.02*

* $P<0.05$ between mean EAAI of groups A and B.

rabbits weaned at 35 days of age, which proved that the age of weaning did not have a significant effect on the biological value of the protein in this muscle.

The higher protein index of LL compared to SM in the rabbits from group B was due to the increased average amount of valine (5.80 and 6.26 g/100 g protein respectively) from the monoamino carboxylic acids, and phenylalanine (3.62 and 4.35 g/100 g protein respectively), from the ring-containing amino acids group. The overall content of leucines was also higher – from 13.62 g/100 g protein in LL to 14.44 g/100 g protein in SM. Leucine LL content was higher by 4.9% and that of isoleucine – by 8.2%, compared to the same amino acids in SM (Table 2).

SM contained more monoamino carboxylic (glutamic acid) and some basic acids (histidine and arginine), which did not have a significant effect on the biological value.

The content of amino acids from the leucines group in LL was higher in group B, compared to group A (means of 13.46 and 14.44 g/100 g protein). Leucine increased from 8.50 to 9.15 g/100 g protein (up by 7.6 %), and isoleucine – from 4.97 to 5.30 g/100 g protein (up by 6.6 %). Phenylalanine content rose from 4.05 to 4.35 g/100 g protein (up by 7.4 %). The content of monoaminocarboxylic acids was also higher, the most significant difference being that between valine LL contents of both groups: 5.46 g/100 g protein for group A and 6.26 g/100 g protein in group B (up by 14.7 %). The higher content of basic amino acids – 19.32 and 20.57 g/100 g protein (up by 6.47 %) also had an effect on the protein's biological value. The content of essential amino acids, histidine and arginine from this group, changed with weaning age from 9.27 to 9.97 g/100 g protein (up by 7.6%) for lysine, 4.03 to 4.37 g/100 g protein (up by 8.4 %), for histidine and 6.02 to 6.23 g/100 g protein (up by 3.5 %) for arginine.

In rabbits weaned on the 21[st] day, there was no considerable difference between the biological value of the proteins

Table 3. Content of essential and non-essential amino acids in rabbit meat protein (g/100 g protein) depending on their weaning age: 21 days (group A; n=7) or 35 days (group B, n=8). Values are presented as mean±SD

	M. Semimembranosus		M. Longissimus Lumborum	
	Group A	Group B	Group A	Group B
Essential amino acids				
Arginine	5.96±0.08	7.02±0.21***	6.02±0.24	6.23±0.16**
Valine	5.35±0.13	5.80±0.11***	5.46±0.20	6.26±0.31***
Isoleucine	4.90±0.10	4.90±0.07	4.97±0.08	5.30±0.15***
Leucine	8.44±0.26	8.72±0.14**	8.50±0.24	9.15±0.22***
Lysine	9.21±0.10	9.78±0.19***	9.27±0.08	9.97±0.18***
Methionine	2.46±0.06	1.98±0.08***	2.46±0.12	2.02±0.16***
Tyrosine	3.24±0.09	2.54±0.15***	3.21±0.07	2.75±0.21***
Threonine	4.24±0.05	3.21±0.14***	4.22±0.06	3.33±0.15
Phenylalanine	3.98±0.12	3.62±0.06***	4.05±0.16	4.35±0.19***
Histidine	3.99±0.20	5.25±0.26***	4.03±0.18	4.37±0.42**
Non-essential amino acids				
Alanine	5.91±0.12	6.07±0.14*	5.92±0.05	6.05±0.26 *
Aspartic acid	9.29±0.05	9.69±0.11***	9.30±0.11	9.68±0.28 **
Glycine	4.86±0.30	5.15±0.41**	4.69±0.12	4.96±0.23 *
Glutamic acid	18.75±0.32	18.63±0.22	18.69±0.32	17.98±1.18**
Proline	5.08±0.29	5.04±0.33	4.97±0.20	4.73±0.45
Serine	3.19±0.10	1.55±0.17***	3.07±0.06	1.75±0.18***
Cysteine	1.15±0.19	1.05±0.17	1.20±0.15	1.13±0.14

* p<0.05; ** p<0.01; *** p < 0.001 between groups A and B.

in the two muscles, yet there was a significant difference for rabbits weaned on the 35th day ($P<0.05$).

Comparing the values of the individual essential amino acids in SM between the two groups, it was established that in normally weaned rabbits lysine, arginine, valine and histidine contents were statistically significantly higher ($P<0.001$) compared to the early weaned group (Table 3). The opposite was true for methionine, tyrosine, threonine and phenylalanine, which had higher levels in early-weaned rabbits ($P<0.001$). In LL meat, only the methionine and threonine contents in group A were significantly higher than those of group B ($P<0.001$). Along with that, the amounts of valine, isoleucine, leucine, lysine, phenylalanine ($P<0.001$), arginine and histidine ($P<0.01$) were greater compared to what was assayed in group A.

Among the non-essential amino acids in SM meat, only the serine content was significantly higher in early-weaned animals, compared to the normally weaned ($P<0.001$). Alanine ($P<0.05$), aspartic acid ($P<0.001$) and glycine ($P<0.01$) were found in significantly higher amounts in the meat from group B. A similar tendency in the values of these three amino acids was observed in the LL meat. Only serine and glutamic acid exhibited significantly higher values in the rabbits from group A. The amino acids proline and cysteine were also determined to have higher yet statistically insignificant values in both muscles from group A.

DISCUSSION

Amino acid content of rabbit meat was discussed and interpreted after their allotment to groups according to their chemical structure: monoamino carboxylic (glycine, alanine, valine); monoamino dicarboxylic (aspartic and glutamic acids); ß-hydroxyamino carboxylic (serine and threonine); basic (lysine, histidine, arginine); sulfur-containing (methionine and cysteine); ring-containing (phenylalanine, tyrosine, proline) and leucines (leucine and isoleucine). This enabled the determination of the relative shares of the groups and the changes in them (Zhelyazkova *et al.* 2006; Zhelyazkova, 2007).

On the basis of the average content of amino acids in the two examined muscles as indicator of meat quality, it could be concluded that the weaning age of rabbits had an effect on the amino acid content, the biological value of proteins, and therefore, on the overall rabbit meat quality. It was shown that the meat of normally weaned rabbits had a higher biological value of proteins than that of the early weaned. This was confirmed by the higher mean values of monoamino carboxylic acids – by 11.7% for valine (from 5.40 g in group A to 6.03 g in group B), as well as of basic amino acids – by 10.8 % (from 19.24 g to 21.31 g). From the group of the basic amino acids, lysine and histidine contents were increased by 6.9 %, and that of arginine – by 10.7% The content of amino acids from the leucines group was also higher (leucine by 5.4 %, and isoleucine – by 3.4 % in group B, compared to group A). These results were confirmed by mean values of these amino acids, which were statistically significantly higher in the meat of rabbits weaned on the 35th day.

Our results are similar to those reported by Grigorov (2008), who reached the conclusion that the meat of rabbits slaughtered at the age of 90 or 120 days had the highest quality with regard to content of essential amino acids per 100 g of protein and exhibited the best essential amino acid index. Pla (2008) demonstrated that the levels of sulfur-containing amino acids (methionine and cysteine) was higher when rabbits were fed an organic diet and weaned on the 30th day, compared to those fed a standard diet and weaned on the 28th day. There were no significant sex-related differences in the amino acid content of meat in both groups.

The amino acid content of lamb m. Longissimus dorsi was with the highest content of lysine, leucine, arginine and valine (Löest *et al.*, 1997). Our results have shown the same progression in both experimental groups. A similar progression in the content of essential amino acids in 100 g protein has been established by a number of authors in goat meat (Ferreira, 2004), poultry meat (Siddiqi *et al.*, 1994), broiler chicken's breast meat (Hamm, 1991). The reverse relationship was reported for glycine, threonine and serine. Examining the influence of pigs' body mass and sex on the amino acid content of the meat, it was established that glutamic and aspartic acids exhibited the highest values in animals slaughtered at 115.1 kg, compared to those with slaughter weights of 105 and 115 kg (Okrouhla *et al.*, 2006). In this study, these two amino acids also had the highest values – aspartic acid in group B and glutamic acid in group A.

Normal weaning of rabbits had a stronger effect on the biological value of LL but not of SM protein. According to Thompson *et al.* (2006) the proteins with EAAI equal to or higher than 0.90 are of high quality, complete proteins have an EAAI of about 0.80, and incomplete – less than 0.70.

In conclusion, the weaning age of rabbits influenced the amino acid content and biological value of muscle proteins. In normally weaned rabbits, proteins exhibited higher values due to the greater proportion of basic amino acids (lysine, histidine, arginine), those from the leucines group (leucine and isoleucine), and the monoamino carboxylic group (valine). Moreover, the biological value of proteins in m. Longissimus Lumborum meat was influenced the most because its values for both groups were higher than those for m. Semimebranosus. In light of this, it can be considered that normal weaning of rabbits on the 35th day is preferable from the point of view of better amino acid content and biological value of the proteins in their meat.

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