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**Address of Editorial office**:
Agricultural Science and Technology
Faculty of Agriculture, Trakia University
Student's campus, 6000 Stara Zagora
Bulgaria
Telephone.: +359 42 699330
+359 42 699446
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**Technical Assistance:**
Nely Tsvetanova
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# Amino acid composition of lamb meat from the North East Bulgarian fine fleece breed and its crossbreds with Australian merino and Ile de France from internal breeding

R. Slavov[1]*, G. Mihaylova[1], St. Ribarski[2], D. Panayotov[1], D. Pamukova[1], D. Dragnev[1]

[1]Department of Animal Science – Ruminants and Dairy Farming, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
[2]Department of Morphology, Physiology and Nutrition, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

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***Abstract.*** *A comparative analysis of amino acid composition of lamb meat from the North East Bulgarian fine fleece breed (I gr.) and its crossbreds from internal breeding with 25% heredity from the Australian merino (II gr.) and Ile de France (III gr.) breeds was conducted. Upon starting the experiment lambs were equal and during the experiment they were placed under similar conditions of feeding and rearing. To establish the amino acid composition of meat slaughter analyses were performed at 100 and 130 days of age. From the carcass of each slaughtered animal individual mean samples were taken. Studies were carried out in the Research Laboratory of the Faculty of Agriculture at Trakia University. As a result of the studies the following conclusions were made: ¼ Ile de France crossbreds in a sophisticated crossing have the highest total amino acid content, including essential amino acids in the meat of 100- and 130-day-old lambs, 6.87% and 7.36%, respectively. The lysine/arginine ratio, relating to protein atherogenicity, varies within narrow ranges among groups and marks slight increase with age – from 1.31-1.37 at 100 days to 1.41-1.46 at 130 days. With the increase of age in crossbreds from internal breeding total protein amino acid content grows, that being most prominent in lambs from the III group – from 41.92 to 43.49%. The values of total protein indices increase compared to the reference protein (from 117.97% to 118.22% for II group and from 116.44% to 120.80% for III group) and the whole egg protein (from 89.39% to 91.92% for II group and from 90.53% to 93.93 for III group). It has been found that internal breeding of crossbreds with 25% heredity from the Ile de France breed has positive effect concerning the total amino acid content of lamb meat at 100 and 130 days of age, essential amino acid content in it and the levels of total amino acid indices. Internal breeding of crossbreds with 25% heredity from the Australian merino breed does not have an adverse effect on the levels of the studied traits.*

**Keywords:** lambs, crossing, internal breeding, meat, amino acid content of meat and amino acid index of meat

## Introduction

The processes of improvement of modern fine fleece sheep breeds aim at applying specific selection approaches for improvement of the traits that are of economic significance for the productive line. Along with increase of wool yield and improvement of the quality traits of wool in fine fleece sheep breeding, meat yield is of particular economic importance both in quantitative and qualitative aspect. For Bulgaria, as well as for other countries with continental climate, it is necessary to use the Australian merino breed as a corrector of the qualitative traits of wool. However, the issue about the effect of heredity from Australian merinos on the quantitative and qualitative characteristics of meat in crossbreds, especially in animals from internal breeding, is not totally clear.

Studies on crossing fine fleece ewes with rams of the Australian merino breed in Russia were carried out by Kravchenko (1988) and Moroz (1993) with the Groznenska breed, Moroz and Ibrahimov (1992) and Zulaev (1992) with Stavropol breed, Zubkov et al. (1995) with Kavkaz breed, Ozhigov (1990) and Galatov (1991) with Soviet merino breed, Danilenko (1989) and Shtompel (1989) with Askaniyska breed, etc. In Bulgaria similar research on crossbreeding of fine fleece ewes with rams from the Australian merino breed are conducted by Stoyanov et al. (1982), Boykovski (1994-1995), Slavov (2007) – with North East Bulgarian fine fleece breed, Tsenkov et al. (1995), Slavova (2000) with the Thracian fine fleece breed, Lazarov and Iliev (1997) Iliev (1999) and– with the Karnobat merino breed, etc.

Lembit (1990) thinks that losses from the decline in the price of wool on the international market could be offset by improving fertility and meat production of the sheep. Live weight, growth intensity, quantitative and qualitative characteristics of meat production determine to a large extent the efficiency in sheep breeding.

In order to enhance economic significance, in a number of fine fleece farms in Bulgaria sophisticated crossing with the Ile de France breed has been started. The resulting crossbreds, and especially the ones from internal breeding, require a thorough assessment of meat yield, both in quantitative and qualitative aspect. Studies of meat yield qualities of lambs of the Ile de France breed and different types of crossbreds with its involvement are carried out by Feige and Lamber (1980), Vosloo (1996), Dimitrov and Ivanov (1986), Chakarov and Marinova (1986), Dimitrov (1988), Raycheva and Ivanova (2005), Slavov (2007), etc.

The nutritive and biologic value of sheep meat depends on its protein content as well, incl. nonessential and essential amino acids. Studies on the amino acid composition of proteins in lamb, hogget and mutton are related primarily to establishing the effect of various factors – breed and type of crossing, age, live weight before slaughtering, nutrition level and ration composition, type of birth, season of lambing, technology of rearing, stress, etc. – Roger and Egan (1974), Crouse (1983), Dimitrov (1988), Slavov (1990,1995), Slavova et al. (2001), Bellof and Pallauf (2004), Oriani et al. (2005), Brzostovski and Tanski (2006), Abd El-aal and Suliman (2008), Peraza-Mercado et al. (2010), Chernukha (2011), Uzakov and Ospakova (2014), Slavov et al. (2015), etc.

The aim of the study was to perform a comparative analysis of amino acid composition of meat from lambs of the North East

* e-mail: rslavov@uni-sz.bg

Bulgarian fine fleece breed and its crossbreds from internal breeding with 25% heredity from the Australian merino and Ile de France breeds – internal breeding.

## Material and methods

The experiment to study the amino acid composition of meat was carried out with 42 lambs divided into 3 groups of 14 animals each - I group – North East Bulgarian fine fleece (NEBFF); II group - ¼-blood crossbreds with Australian merino (AM) – internal breeding - ¼ AM IB - ♀¼ AM (♀NEBFF x ♂½ AM) x ♂¼AM (♀NEBFF x ♂½ AM) and III group - (♀NEBFF x ♂½ AM) – blood crossbreds with Ile de France (IIF)– internal breeding - ¼IIF IB - ♀¼IIF (♀NEBFF x ♂ ½IIF) x ♂¼IIF (♀NEBFF x ♂¼IIF).

The experiment was carried out on the territory and with the animals owned by Dobrudzha Agricultural Institute – town of General Toshevo, Dobrich area. Upon starting the experiment lambs were made equal by age (44-46 days old), gender (equal number of males and females), type of birth (equal number of singles and twins), age of weaning (33 – 36 days), age of mothers and number of lambings (3 yers - II lambing).

During the experiment the lambs were placed under equal conditions of raising and subjected to intensive fattening until they were 130 days old. Feeding is ad libitum, with starter mixture for fattening lambs containing per 1 kg 1.22 FUG (6.72 MJ), 106.1 g PDI, lucerne hay containing per 1 kg 0.61 FUG (3.12 MJ) and 79.9 g PDI, as well as corn silage containing per 1 kg 0.30 FUG (1.68 MJ) and 19.9 g PDI (Todorov and Dardzhonov, 1995).

The control of live weight of the experimental lambs was made at 14- dayinterval, with an accuracy of 100 g. Feed consumption was recorded daily and by periods. To establish the quantitative and qualitative traits of meat yield slaughter analyses were carried out – at 100 and 130 days of age, 4 lambs being slaughtered from each group with live weight close to the average for the groups. The slaughtering was carried out according to the requirements of Ordinance No. 27 (promulgated in SG No. 99/1999). The cutting of carcases was carried out by methods applied in INRA.

Pooled samples of meat were taken from the carcass of each slaughtered animal for amino acid analysis. Studies were conducted in the Research laboratory at the Faculty of Agriculture University of Trakia University. Modern equipment was used and standard methods applied. Amino acid composition of meat was carried out by the ion exchange column chromatography method, by acid hydrolysis of the sample in 6 n solution of hydrochloric acid at 110ºC for 24 hours. The residue is dissolved with buffer with pH – 2.2. The Sulphur containing amino acids (methionine and cystine) were determined after oxidation of the sample with mixture of hydrogen peroxide and performic acid. Separation of amino acids (except tryptophan) was carried out with amino acid analyser (AminoAcid Analyser T 339M, Microtechna – Praha) and their quantity was determined by their alloelution volume and standard solution. The results obtained were processed statistically by software "Statistica for Windows" 2010 and the graphics were processed in Excel.

## Results and discussion

In the meat of 100-day old lambs from the studied groups the highest is the amount of glutaminic acid and the lowest is that of the amino acid cystine (Tabel 1). The differences about the content of various amino acids in lamb meat by groups are in most cases mathematically proven. The highest values about amino acid content has lamb meat from the III group – ¼ IIF internal breeding except for phenylalanine content. The lysine/arginine ratio that is related to protein atherogenicity is almost equal for lamb meat in all three groups – 1.31 – 1.37.

The total amount of nonessential and essential amino acids in meat, which are an indicator for its biological full-bodiness in the studied lamb groups at 100 days' age is the highest in lambs from III group – 18.07%. Their meat has the highest essential amino acid content – 6.87% and non-essential amino acid content – 11.2%, followed by the meat of purebred lambs (I group), with total amino acid content – 17.56% and, respectively. essential amino acid content – 6.63% and non-essential amino acid content – 10.93%. The meat of lambs from II group occupies intermediary position (Figure 1).

The results of the study show that at that age in all three groups the most deficient in comparison to the reference protein is the amount of amino acids methionine + cystine. In comparison to the egg protein again the most deficient are methionine and cystine. The chemical index of meat from the studied groups varies from 58.49% for III group; 59.06 for I group, to 5925 for II group (Table 2). Apart from the amount methionine + cystine concerning the egg protein lower are the values of the following amino acids: threonine, leucine, isoleucine and valine. Only the lysine content and the amount of phenylalanine and tyrosine exceed the egg protein values.

The highest is the essential amino acid content in 100 g total protein – 41.92 g and the highest average indices in comparison to the reference protein – 116.44% and to the egg protein – 90.53% has lamb meat from III group. With the lowest values on these parameters is lamb meat from II group and that of purebred lambs has intermediary position.

Amino acid content in the meat of lambs aged 130 days, (except for glycine), is the highest in lamb meat from III group, followed by that of meat from the animals from II group. The lowest are the values in I group. The differences among groups in most cases are mathematically proven (Table 1). The lysine/arginine ratio varies within narrow boundaries – 1.41 – 1.46.

The total amount of non-essential and essential amino acids varies within 18.60% in III group and 17.17% in I group. The highest is the essential amino acid content in lamb meat from III group – 7.36%, and the lowest that of I group – 6.54%. Lamb meat of II group occupies intermediary position (Figure 2).

The values of the protein amino acid index in lamb meat at 130 days (Table 2) show that limiting amino acid compared to the reference protein in all studied groups is the amount of methionine + cystine with amino acid index of 87.71% in II group, 91.14% in I group and 95.43% in lamb meat of III group.

In comparison to the egg protein again the most deficient is the amount of methionine + cystine, the indices varying from 57.92% in II group to 63.02% in III group. Deficient in comparison to the egg protein are the other amino acids as well, except for lysine and phenylalanine + tyrosine.

The highest is the essential amino acid content in 100 g total protein (43.49%) and the highest average indices in comparison to the reference protein (120.80%) and in comparison to the egg protein (93.93%) has lamb meat from III group. The lowest values on these parameters has lamb meat from II group and that of the purebred ones occupies intermediary position.

Taking into account the effect of age (from 100 to 130 days) we

**Table 1.** Amino acid content in 100 g meat of lambs at 100 and 130 days

| Amino acids | Age 100 days | | | | | | Age 130 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I Group | | II Group | | III Group | | I Group | | II Group | | III Group | |
| | MEAN ± SEM | CV | MEAN ± SEM | CV | MEAN ± SEM | CV | MEAN ± SEM | CV | MEAN ± SEM | CV | MEAN ± SEM | CV |
| **Essential amino acids** | | | | | | | | | | | | |
| Lysine | 1.56 $^{xx}$ ± 0.041 | 5.31 | 1.44$^{xx}$ ± 0.037* | 5.31 | 1.60$^{xx}$ ± 0.030** | 3.79 | 1.56$^{xx}$ ± 0.061 | 7.79 | 1.66 ± 0.081* | 3.82 | 1.76$^{xx}$ ± 0.074** | 8.39 |
| Threonine | 0.80$^{xxx}$ ± 0.011*** | 2.86 | 0.76$^{xxx}$ ± 0.021* | 2.86 | 0.82$^{xx}$ ± 0.012** | 2.88 | 0.67$^{xx}$ ± 0.037*** | 1.09 | 0.78 $^{x}$ ± 0.031* | 8.40 | 0.78$^{xx}$ ± 0.038** | 9.74 |
| Valine | 0.89$^{xx}$ ± 0.015** | 3.35 | 0.86$^{xxx}$ ± 0.017*** | 3.35 | 0.93$^{xx}$ ± 0.014*** | 3.06 | 0.94$^{xxx}$ ± 0.023** | 4.87 | 0.99$^{xxx}$ ± 0.016*** | 3.19 | 1.05$^{xxx}$ ± 0.032*** | 6.00 |
| Methionine | 0.39$^{xxx}$ ± 0.023* | 11.77 | 0.37$^{xxx}$ ± 0.018 | 11.77 | 0.42$^{xxx}$ ± 0.011** | 5.27 | 0.38$^{xxx}$ ± 0.019* | 10.08 | 0.37$^{xxx}$ ± 0.026 | 14.14 | 0.44$^{xxx}$ ± 0.011** | 5.07 |
| Isoleucine | 0.81$^{xxx}$ ± 0.015* | 3.70 | 0.77$^{xxx}$ ± 0.024*** | 3.70 | 0.84$^{xxx}$ ± 0.018*** | 4.23 | 0.83$^{xxx}$ ± 0.037* | 8.96 | 0.89$^{xx}$ ± 0.017*** | 3.82 | 0.94$^{xxx}$ ± 0.034*** | 7.21 |
| Leucine | 1.43$^{xxx}$ ± 0.031 | 4.28 | 1.38$^{xxx}$ ± 0.031** | 4.28 | 1.49$^{xxx}$ ± 0.029* | 3.84 | 1.41$^{xxx}$ ± 0.043 | 6.04 | 1.49$^{xx}$ ± 0.026** | 3.46 | 1.57$^{xxx}$ ± 0.056* | 7.15 |
| Phenylalanine | 0.75$^{xxx}$ ± 0.017 | 3.12 | 0.79$^{xxx}$ ± 0.013 | 3.12 | 0.77$^{xx}$ ± 0.031* | 7.92 | 0.75$^{xx}$ ± 0.032 | 8.71 | 0.78$^{x}$ ± 0.040 | 10.25 | 0.82$^{xx}$ ± 0.050* | 12.28 |
| **Non-essential amino acids** | | | | | | | | | | | | |
| Histidin | 0.68$^{xxx}$ ± 0.023** | 6.69 | 0.65$^{xxx}$ ± 0.014*** | 6.69 | 0.75$^{xxx}$ ± 0.012*** | 3.19 | 0.74$^{xxx}$ ± 0.028** | 7.40 | 0.81$^{xx}$ ± 0.021*** | 5.23 | 0.86$^{xxx}$ ± 0.021*** | 4.74 |
| Arginin | 1.15 ± 0.030** | 5.27 | 1.10$^{xxx}$ ± 0.017* | 5.27 | 1.16$^{xxx}$ ± 0.022* | 3.79 | 1.11$^{xx}$ ± 0.017** | 9.12 | 1.16$^{x}$ ± 0.016* | 2.72 | 1.20$^{xx}$ ± 0.050* | 8.20 |
| Aspatic acid | 1.60$^{xxx}$ ± 0.029 | 3.61 | 1.55$^{xxx}$ ± 0.033*** | 3.61 | 1.67$^{xxx}$ ± 0.031* | 3.64 | 1.61* ± 0.038 | 4.76 | 1.70 ± 0.036*** | 4.19 | 1.75$^{x}$ ± 0.082* | 9.28 |
| Serine | 0.61$^{xx}$ ± 0.015*** | 4.92 | 0.58$^{xxx}$ ± 0.014*** | 4.92 | 0.62$^{xxx}$ ± 0.008** | 2.41 | 0.46$^{xxx}$ ± 0.034*** | 19.63 | 0.50$^{xx}$ ± 0.040*** | 16.02 | 0.55$^{xxx}$ ± 0.029** | 10.53 |
| Glutamic acid | 3.14 ± 0.059* | 3.72 | 3.08$^{x}$ ± 0.073* | 3.72 | 3.18$^{x}$ ± 0.063* | 3.94 | 3.05$^{xx}$ ± 0.074* | 4.86 | 3.21$^{x}$ ± 0.049* | 3.10 | 3.25$^{xx}$ ± 0.047* | 9.03 |
| Proline | 0.95$^{xxx}$ ± 0.029* | 6.02 | 0.94$^{xxx}$ ± 0.036** | 6.02 | 1.01$^{xxx}$ ± 0.039** | 7.66 | 0.91 ± 0.046* | 10.05 | 0.88$^{xx}$ ± 0.014** | 3.11 | 0.91$^{xx}$ ± 0.027** | 5.93 |
| Glycine | 1.01 ± 0.069 | 13.62 | 1.02$^{xx}$ ± 0.024** | 13.62 | 0.98$^{xx}$ ± 0.035** | 7.21 | 1.03$^{xxx}$ ± 0.023 | 23.90 | 0.89$^{xxx}$ ± 0.035** | 7.82 | 0.87$^{xxx}$ ± 0.023** | 5.23 |
| Alanine | 1.08$^{xxx}$ ± 0.026 | 4.84 | 1.06$^{xxx}$ ± 0.017** | 4.84 | 1.11$^{xxx}$ ± 0.011 | 1.99 | 1.09$^{xx}$ ± 0.027 | 4.92 | 1.08$^{x}$ ± 0.013** | 2.30 | 1.11$^{xx}$ ± 0.030 | 5.79 |
| Cysteine | 0.17$^{xx}$ ± 0.005 | 6.27 | 0.17$^{xx}$ ± 0.006 | 6.27 | 0.18$^{xx}$ ± 0.015 | 16.33 | 0.17 ± 0.008 | 9.59 | 0.17$^{xx}$ ± 0.012 | 13.60 | 0.18$^{xx}$ ± 0.011 | 12.13 |
| Tyrosine | 0.54$^{xx}$ ± 0.023* | 8.43 | 0.52$^{xx}$ ± 0.015 | 8.43 | 0.54$^{xx}$ ± 0.005** | 1.77 | 0.46$^{xxx}$ ± 0.045* | 19.54 | 0.48$^{xx}$ ± 0.035 | 14.54 | 0.56$^{xxx}$ ± 0.015** | 5.25 |

Reliability among groups by age $^{x}$ at p<0.05 $^{xx}$ at p<0.01 $^{xxx}$ at p<0.001, Reliability among groups with change of age *at p<0,05 **p<0,01 *** p<0,001

**Table 2.** Amino acid indices of meat protein of lambs

| Essential amino acids | Reference protein g/100g | Whole-egg protein g/100g | I Group g/100g total protein | I Group Indices: Compared to refer. protein, % | I Group Indices: Compared to egg protein, % | II Group g/100g total protein | II Group Indices: Compared to refer. protein, % | II Group Indices: Compared to egg protein, % | III Group g/100g total protein | III Group Indices: Compared to refer. protein, % | III Group Indices: Compared to egg protein, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| At 100 days | | | | | | | | | | | |
| Threonine | 4.0 | 4.8 | 4.57 | 114.25 | 95.21 | 4.51 | 112.75 | 93.96 | 4.58 | 114.50 | 95.42 |
| Leucine | 7.0 | 8.8 | 8.16 | 116.57 | 92.73 | 8.09 | 115.77 | 91.93 | 8.24 | 117.71 | 93.64 |
| Isoleucine | 4.0 | 6.7 | 4.61 | 115.25 | 68.81 | 4.55 | 113.75 | 67.91 | 4.67 | 116.75 | 69.70 |
| Valine | 5.0 | 7.2 | 5.10 | 102.00 | 70.83 | 5.07 | 101.40 | 70.42 | 5.19 | 103.80 | 72.08 |
| Methionine+Cystine | 3.5 | 5.3 | 3.13 | 89.43 | 59.06 | 3.14 | 89.71 | 59.25 | 3.10 | 88.57 | 58.49 |
| Lysine | 5.5 | 6.2 | 8.88 | 161.45 | 143.23 | 8.76 | 159.27 | 141.29 | 8.86 | 161.09 | 142.90 |
| Phenylalanine+Tyrosine | 6.0 | 5.7 | 7.28 | 121.33 | 127.72 | 7.27 | 121.17 | 127.54 | 7.28 | 121.33 | 127.72 |
| Tryptophan | 1.0 | 1.6 | - | - | - | - | - | - | - | | - |
| Total | 36.0 | 46.3 | 41.73 | 115.91 | 90.12 | 41.39 | 114.97 | 89.39 | 41.92 | 116.44 | 90.53 |
| At 130 days | | | | | | | | | | | |
| Threonine | 4.0 | 4.8 | 3.92 | 98.00 | 81.67 | 4.17 | 104.25 | 86.87 | 4.18 | 104.50 | 87.08 |
| Leucine | 7.0 | 8.8 | 8.25 | 117.86 | 93.75 | 8.41 | 120.14 | 95.57 | 8.45 | 120.71 | 96.02 |
| Isoleucine | 4.0 | 6.7 | 4.84 | 121.00 | 72.24 | 5.02 | 125.50 | 74.92 | 5.06 | 126.50 | 75.52 |
| Valine | 5.0 | 7.2 | 5.47 | 109.40 | 75.97 | 5.57 | 111.40 | 77.36 | 5.63 | 112.60 | 78.19 |
| Methionine+Cystine | 3.5 | 5.3 | 3.19 | 91.14 | 60.19 | 3.07 | 87.71 | 57.92 | 3.34 | 95.43 | 63.02 |
| Lysine | 5.5 | 6.2 | 9.11 | 165.64 | 146.94 | 9.34 | 169.82 | 150.65 | 9.47 | 172.18 | 152.74 |
| Phenylalanine+Tyrosine | 6.0 | 5.7 | 6.93 | 115.50 | 121.58 | 6.98 | 116.33 | 122.46 | 7.36 | 122.67 | 129.12 |
| Tryptophan | 1.0 | 1.6 | | - | - | - | - | - | - | - | - |
| Total | 36.0 | 46.3 | 41.71 | 115.86 | 90.08 | 42.56 | 118.22 | 91.92 | 43.49 | 120.80 | 93.93 |

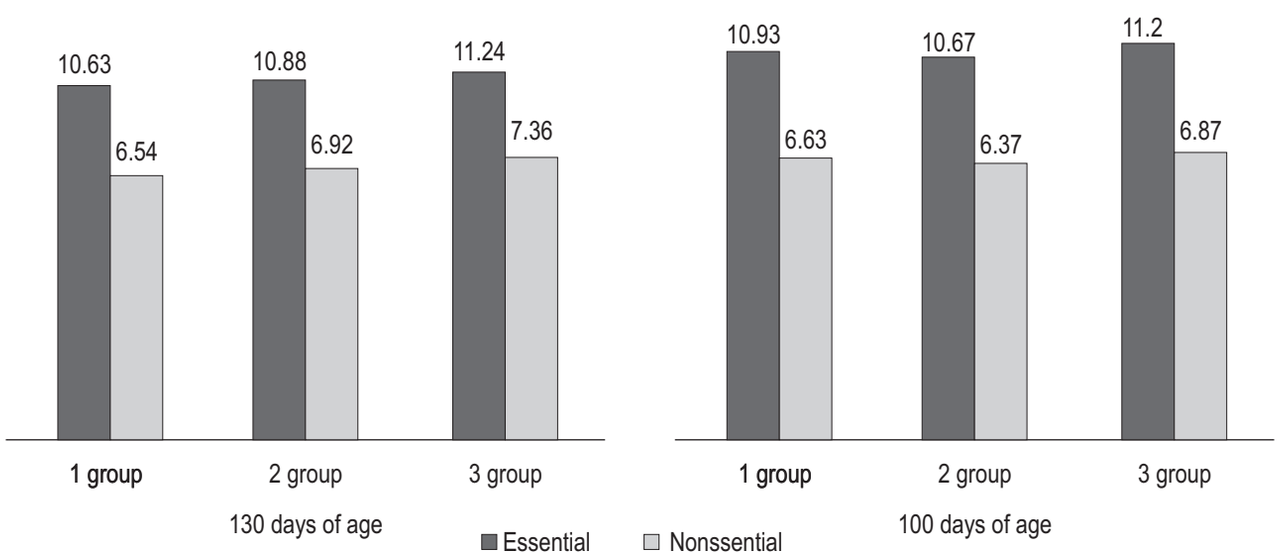


**Figure 1.** Essential and nonessential amino acids in meat, %

find out that with its increase there are changes in the amino acid content of lamb meat by groups, the differences in the majority of cases being mathematically proven (Table 1). With age increase of both the total amino acid content in II and III group (Figures 1 and 2), and essential amino acid content is found, their values being the highest in III group – 6,87% at 100 days' age and 7,36% at 130 days, respectively.

## Conclusions

Results obtained give reason to the following:

- ¼ IIF crossbreds have the highest total amino acid content and essential amino acid content in meat at the age of 100 and 130 days, 6.87% and 7.36%, respectively.
- The lysine/arginine ratio being related to protein atherogenicity, varies within narrow boundaries among groups and increases slightly with age – from 1.31 to 1.37 at the age of 100 days and from 1.41 to 1.46 at the age of 130 days.
- With the increase of age in crossbreds from internal breeding amino acid content in the total protein increases, that being the most pronounced in lambs from III group – from 41.92% to 43.92%
- With the increase of age in crossbreds from internal breeding total protein increases – from 114.97% to 118.22% for II group and from 116.44% to 120.80% in III group. In purebred animals age-related changes are insignificant.
- Internal breeding of crossbreds with 25% heredity from the Ile de France breed has positive effect on the total amino acid content in the meat of lambs aged 100 and 130 days, its essential amino acid content and the level of total amino acid indices.
- Internal breeding of crossbreds with 25% heredity from Australian merino does not have an adverse effect on the levels of the studied traits.

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***Instruction for authors***

**Preparation of papers**

Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper ( including abstract references, tables, figures and other appendices)

**The manuscript** should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

**The title** needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

**Names and affiliation of authors** The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

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**Results** are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. **Tables** should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

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**Discussion:** The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

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