RESEARCH REGARDING THE BIOLOGICAL VALUE OF MEAT PROTEIN FROM SHEEP

Otilia Cristina Crăciun[1*], Roxana Lazăr[1], P.C. Boişteanu[1]

[1] *University of Agricultural Sciences and Veterinary Medicine Iasi, Romania*

Abstract

Through this paper aims the objective of assessment the quality of meat from slaughtered sheep at different ages, through the protein content of different types of muscles. Qualitative analysis involves, besides the structural proteins determining in addition to evaluating content in essential amino acids and amino acid nitrogen content and the pH determination after slaughter, colour and textural properties (Warner Bratzler) of meat. Biological material was represented by Longissimus dorsi muscles and Triceps brachii, collected from lambs and sheep slaughtered in the slaughterhouse. To determine the amino acid was used HPLC chromatographic method, meat colour was analyzed using spectrophotometer, meat acidity with pH meter and meat tenderness by texturometer. Biological studies have highlighted the superior nutritional characteristics of sheep meat. The statistical interpretation of data for proteins in amino acid content reveals significant differences ($p<0.001$) according to the muscle; histidine, arginine, valine and isoleucine presenting in Longissimus dorsi muscle superior value (23.82 mg/g, 55.76 mg/g, 38.92 mg/g and 35.23 mg/g) compared to amino acids values recorded for Triceps brachii (1.29 mg/g, 46.68 mg/g, 35.79 mg/g and 31.84 mg/g). Brachial triceps muscle analysis revealed significant differences by age (lambs showing lower values of histidine 1.29 mg/g to 18.93 mg sheep/g).

Key words: sheep meat, physico-chemical and quality proprieties

INTRODUCTION

Protein is essential for growth and development providing the body with energy and needed for the manufacture of hormones, antibodies, enzymes and tissues [4]. It also helps maintain the proper acid-alkali balance in the body.

When consumed, it is broken down into amino acids, the building blocks of all proteins. Some of which are designated nonessential. Humans can produce ten of the twenty amino acids. The others must be supplied in the diet daily because the human body does not store excess amino acids for later use, unlike fat and starch. Failure to obtain enough of even one of the ten essential amino acids; result in degradation of the body's proteins (muscle and other tissues) to counteract the imbalance. The nutritional value or quality of structurally different proteins varies and is governed by amino acid composition, ratios of essential amino acids, susceptibility to hydrolysis during digestion, source and the effects of processing [14]. To optimize the biological utilization of proteins, a better understanding is needed of the various interrelated parameters that influence their nutritive value.

Therefore, the importance of analyzing different sources of consumed protein as meat and as mentioned further on, has not yet been studied for Karakul breed of ovine.

Sheep meat is considered an appreciated food animal for people food, which is a growing worldwide; because sheep can survive, reproduce and produce meat and/or milk in heavy medium.

MATERIAL AND METHODS

The analyses were performed in Physiology Research Laboratory at the Faculty of Animal Science, Iaşi and at the Department of Agricultural research and environmental studies at the University of Udine, Italy.

Experimental measurements were performed on 20 sheep, Karakul breed, males

*Corresponding author: otilia_craciun_ro@yahoo.com
The manuscript was received: 24.04.2012
Accepted for publication: 11.05.2012

of different ages: 10 lambs (4-6 weeks, 7-10 kg carcass weight) and 10 adults (12-14 months, 18-20 kg carcass weight).

After slaughter, carcasses were stored at room temperature (10-15^{0}C) for 6 hours before being refrigerated. Samples were collected from the right side of carcasses after they were chilled at 2-4^{0}C for 4 hours for *Longissimus dorsi* m. is a section performed between the 10th and 11th thoracic vertebra and between the 4 and the 5th lumbar vertebra and the *Triceps brachii* m. section was made between the penultimate and last lumbar vertebra and between the tibiae-femoral joint.

Physical and chemical analyzes were performed on *Longissimus dorsi* m. and *Triceps brachii* m. at 24 hours after slaughter:

- determination of meat acidity with pH meter with electrode Crison 52-32;

- assessment of meat colour in muscle samples with thickness of 1.5 to 2.5 cm, the cut surface after oxygen for an hour, with Spectrophotometric Minolta CM-2600 (system L *, a *, b *), is set to view the beam angle of 10^0 standard illuminate D65 in colorimetric space CIE Lab. The measurement itself was done in three different areas of each muscle sample at room temperature of 15^{0}C. The results were converted and processed using software SpectraMagic. The calibration of chromo-meter was performed before each series of measurements with calibration device Minolta CM-A32, this based on a "black standard" and "white standard".

- after 4 days of maturation at 2-4^{0}C were determined: rate of water loss by boiling (WHC) in the hot water at 75^{0}C for 20 minutes and shear strength using dynamometer testing device accompanied by the forces of cutting Warner Bratzler (WBS) [1].

The samples subjected to heat treatment (in polyethylene bag), then wrapped in aluminium foil, stored for 24 hours at 2^{0}C, were sectioned in a cylindrical (3-cylinder with a diameter of 1.5 cm and length 2 cm) in the muscle fibbers sense. Force was used to determine the specific blade (angle of 60^0, speed 100 mm/min, cutting force 1000 N) attached on Lioyd Plus TA Instruments texture-meter.

The cylindrical muscle samples were sectioned in the perpendicular direction to muscle fibbers; maximum force indicator stalk test was intended to describe the meat tenderness. Integrated software NEXYGEN Ondio on the texture-meter Series TaPlus allowed direct calculation of the shear forces values by cutting-deformation curve as required by BS EN ISO 7500:1999 [13]. Each physicochemical measurement was performed three repetitions.

These characteristics were evaluated in relation to age and muscle region of subjects belonging Karakul breed, who had the same system of growth and nutrition in Horleşti farm, from Iaşi.

To determine the total amino acid, sulphur and tryptophan content, samples *Longissimus dorsi* and *Triceps brachii* were frozen at -18^{0}C after having first been wrapped in polyethylene film in vacuum atmosphere, chopped, and then dried: liquid material was frozen at-50^{0}C, the vacuum pump created a vacuum to 500 atm, primary lyophilisation lasted 5-6 hours and desorption was performed at a temperature of 35^{0}C for 12 hours. Samples were subjected to freeze such rebalancing fluid (keeping them in the air for 12 hours) and chopped until obtain a fine powder.

Preparation of samples: for amino acids determination was used acid hydrolysis except cystine and methionine, which was acid oxidation performance, followed by acid hydrolysis.

Acid hydrolysis: 100 mg were weighed from each sample in a Pyrex glass tube which is mounted a teflon-lined screw cap. In the tubes were added 10 ml of 6M HCl then removed the air from the tube with nitrogen for one minute.

Then hydrolysis was performed for 22-24 hours at 110^{0}C, after which tubes were left to cool at room temperature and it were added 10 ml of internal standard (L-α-amino acid n-butyric acid 2.5 mM in HCl 0.1 M), the contents are collected in a volumetric flask of 250 ml (or 200), then diluted with UHQ. The solution was filtered bottles were collected for derivation.

Performance acid oxidation: abut 50-70 mg of sample was accurately weighed into

a Pyrex glass tube fitted with teflon-lined screw cap were then placed on ice bath for 30 minutes. After adding 2 ml performic acid, samples were kept in an ice bath for 16 hours. Then 0.3 ml of hydrobromic acid were added to remove excess of performic acid and after 30 minutes a vacuum system was used to remove the bromine formed during the reaction. Oxidized samples were hydrolyzed with 6M HCl.

Derivation: Ten micro litres of filtered hydrolysed sample were transferred to a 1.5 ml vial with Teflon-lined screw cap, 70 µl of borate buffer were added, because the optimal pH range for derivation is 8.2 – 9.7, and the solution was briefly vortexes. Then, 20 µl of reconstituted AccQ Fluor reagent (3 mg/ml in acetonitrile) was added and the mixture was immediately vortexes for several seconds. The vial was closed and left to stand for one minute at room temperature. It was then heated in a heating block at 55^0C, for 10 min., derivatives were stable at room temperature for up to one week.

Before being used sample vials and Pyrex tubes were washed with HCl 6 M then rinsed with UHQ water. After this process are released primary and secondary amino acids, making fluorescent derivatives.

Chromatographic conditions: The column was thermostatic at 37°C and the flow rate was 0.8 ml/min. The injection volume was 20 µl.

Mobile phase A consisted of acetate-phosphate aqueous buffer, mobile phase B was acetonitrile 100% and C was UHQ water.

Before beginning the gradient, the column was equilibrated in 100% A for 10 min. After the last analysis of the day, the column was washed for 30 min. with 100% UHQ water, then conditioned for 15-20 min. at acetonitrile/water (60:40). If the column has to be stored for more than 72 h, it was kept in 100% of acetonitrile. Detection was carried out by UV-Vis detector at 248 nm and by fluorescence (λ EX 250 nm, λ EM 395 nm).

RESULTS AND DISCUSSIONS

Qualitative analysis and physico-chemical determination of structural proteins was involved in evaluating amino nitrogen content, pH, colour, shear force and cooking losses. The results obtained for raw and prepared meat by boiling are presented in Table 1.

Table 1 Physical properties of sheep meat (lambs and adult)

Study parameters	*Longissimus dorsi* muscle		ANOVA	*Triceps brachii* muscle		ANOVA		
	lambs	adult sheep	F: age	lambs	adult sheep	F: age	Lambs F: m.r.	Sheep F: m.r.
pH 12 h	5.69±0.06	5.84±0.01	***	5.64±0.08	5.84±0.01	***	i.s.	i.s.
L*	35.4±6.01	44.9±2.12	**	37.48±2.4	45.2±0.9	***	i.s.	i.s.
a*	11.06±2.6	18.9±0.9	***	10.23±1.5	18.2±0.9	***	i.s.	i.s.
b*	15.61±1.1	1.4±0.6	***	15.28±1.9	0.75±0.6	***	i.s.	***
C	19.22±2.3	10.75±0.9	***	18.44±2.3	12.73±1.6	**	i.s.	*
H^0	55.23±5.3	66.86±1.8	***	56.34±2.4	74.36±2.7	***	i.s.	***
WBSF (N)	16.2±5.07	36.3±7.89	***	20.8±4.04	27.9±0.11	***	i.s.	***
WBS energy(J)	0.16±0.04	0.36±0.05	***	0.19±0.03	0.27±1.6	***	i.s.	***
Boiling losses %	70.74±8.6	65.85±0.6	i.s.	66.5±2.4	65.43±0.8	i.s.	i.s.	i.s.

F = factor; m.r. = muscle region; L = luminosity; a* = red-green colour coordinated; b* = yellow-blue colour coordinated; C = saturation; h^0 =colour tint; WBSF = Warner Bratzler shear forces; i.s.= insignificant differences; * = significant differences (p<0.05); ** = significant distinct differences (p<0.01); *** = very significant differences (p<0.001).*

Table 2 Composition of the essential and nonessential amino acids (g AA/g sample) of *Longissimus dorsi* and *Triceps brachii* muscles from lambs and adult sheep carcasses

Study parameters	*Longissimus dorsi* muscle		ANOVA	*Triceps brachii* muscle		ANOVA		
	lambs	adult sheep	F: age	lambs	adult sheep	F:age	Lambs F: m.r.	Sheep F: m.r.
Amino nitrogen	8.65±0.26	8.1±0.3	i.s.	7.6±0.4	7.55±0.16	i.s.	i.s.	i.s.
Essential AA Arginine	55.76±0.3	52.7±3.7	i.s.	46.6±3.3	51.4±2.2	i.s.	*	i.s.
Histidine	23.82±1.9	19.8±1.2	i.s.	1.29±0.008	18.9±0.8	***	***	i.s.
Isoleucine	38.07±0.5	35.2±0.2	***	36.4±3.1	31.8±0.8	i.s.	i.s.	*
Leucine	60.2±1.3	57.3±1.02	i.s.	59.7±5.8	53.3±2.09	i.s.	i.s.	i.s.
Lysine	66.69±1.4	65±3.5	i.s.	65.4±2.5	59.93±2.6	i.s.	i.s.	i.s.
Phenylalanine	30.07±1.1	28.5±0.6	i.s.	25.03±1.8	27.18±0.3	i.s.	i.s.	i.s.
Treonine	32.3±0.9	30.1±0.8	**	31.9±1.3	28.2±0.6	**	i.s.	**
Valine	42.5±0.9	38.9±0.3	*	41.4±3.9	35.8±0.8	i.s.	i.s.	*
Tryptophan	44.27±0.9	52.9±0.9	***	116.9±0.9	31.43±0.9	***	***	***
Methionine	21.05±0.9	19.8±0.9	*	15.01±0.9	16.4±0.9	**	***	**
Non essential AA: Cystine	13.82±0.9	10.28±0.9	**	11.9±0.9	11.76±0.8	i.s.	**	**
Alanine	42.8±0.02	39.4±0.3	***	43.9±4.03	37.6±1.5	**	i.s.	**
Aspartic acid	68.5±3.4	65.04±4.2	i.s.	69.8±5.4	58.64±1.2	**	i.s.	*
Glutamic acid	114.4±4.8	115.3±5.7	i.s.	111.9±9.2	103.9±1.8	i.s.	i.s.	**
Glycine	37.4±0.2	31.69±0.6	***	33.8±3.4	37.4±0.2	*	*	***
Proline	30±0.4	28.1±0.1	***	30.3±2.2	28.1±0.8	i.s.	i.s.	i.s.
Serine	25.6±2.06	24.4±1.6	i.s.	25.4±1.3	24.06±0.4	*	i.s.	i.s.
Tyrosine	21.03±3.3	22.2±0.3	i.s.	0.73±0.009	19.09±0.5	***	***	***

AA = amino acids; F = factor; m.r. =muscle region; i.s. =insignificant differences; * = significant differences ($p<0.05$); ** = significant distinct differences ($p<0.01$); *** = very significant differences ($p<0.001$)

At 12 hours after slaughter, pH value reached 5.64 in the *Triceps brachii* muscles from lambs (one month), which are lower than those recorded for adults (14 months), respectively, 5.84 for both muscle regions. Smith and Dobson (1990) [10] pointed out that pH values bring near 5.6 to 12 hours are associated with a stronger light meat. In the present study pH values in relation to muscle region showed significant differences ($p< 0.05$), however, compared to age there were very significant differences ($p<0.001$), muscles harvested from adult male showing a decrease slower of pH (5.84) than those harvested from lambs (5.64), which reduces exudative problems.

Fresh meat colour and its changes are influenced by the concentration and chemical state of myoglobin in muscle pigments and to a lesser extent the presence of haemoglobin [8]. Myoglobin concentration varies according to age and muscle region. Factor "age" for the *Longissimus dorsi* muscle affected redness indices (a *), yellow-blue (b *), saturation (C) and colour tint (h^0) recorded very significant differences ($p<0.001$) between the two age groups. Meat harvested from young adults compared to that of the two muscle regions showed higher levels of muscle myoglobin and total haemal pigment, with a darker colour (higher L * 44.9 vs. 35.4 and 45, 2 vs. 37.4) and red (higher values of a* index: 18.9 vs. 11.06 and 18.2 vs. 10.23).

For *Triceps brachii* muscle age involved recording very significant differences ($p<0.001$) for the L *, a *, b * and h^0 parameters. The statistical analysis performed on the factor "muscle region" have very significant result ($p<0.001$) for the b* and h^0 parameters, in terms of higher average values in *Longissimus dorsi* muscle recorded for the index b * (1.4 vs. 0.75 *Triceps brachii*) and recorded higher mean values of *Triceps brachii* muscle for index h^0 (74.36 vs. 66.86 in *Longissimus dorsi*).

Meat harvest from adult sheep tends to be more tough than that taken from lambs (WBSF: for *Longissimus dorsi* 36.3 N/cm^2 vs. 16.2 N/cm^2 and for *Triceps brachii* 27.9 N/cm^2 vs. 20.8 N/cm^2) recorded very significant differences ($p<0.001$) by age. Similar results were obtained for meat tenderness from adult sheep muscle by region ($p<0.001$), where the *Longissimus dorsi* muscle recorded higher values (36.3 N/cm^2 vs. 27.9 N/cm^2 in *Triceps brachii*). Meat tenderness in lambs showed significant differences ($p<0.05$) by muscle region.

Publications on possible changes in amino acid content of muscle tissue on sheep during breeding are relatively small [7]. Gilka et al. (1989) cited by Loest (1997) [5, 7] says about the protein muscle composition that is genetically determined, and therefore is not expected to change in different growth conditions (e.g. quality and quantity of diet or health). On the other hand, we cannot ignore the fact that the proportion of muscle and tissues may vary according to age [12]. This factor, and muscle region appear to have influenced the amino acid content of sheep carcasses.

In this study the most prominent amino acids analyzed (1 mg AA/g sample) of sheep meat were glutamic acid, aspartic acid, lysine, leucine, arginine and valine for both age categories and muscle regions (Table 2).

Following statistical analysis of the protein content in amino acid results for tryptophan show very significant differences ($p > 0.001$) according for both age categories and muscle region. Thus, *Longissimus dorsi* muscle recorded higher average values of tryptophan in adult sheep (52.9 mg AA/g in sheep vs. 44.27 mg AA/g in lambs) as opposed to *Triceps brachii* muscle which was recorded higher average amount in lambs (116.9 mg AA/g vs. 31.4 mg AA/g in sheep). Comparing the values obtained from tryptophan according to the muscle region, lambs revealed significant differences ($p<0.001$), *Triceps brachii* muscle holding higher average amounts quantities (116.9 mg AA/g) towards *Longissimus dorsi* muscle (44.2 mg AA/g). However, lambs studied, had lower average amounts of histidine in the *Triceps brachii* m. (1.29 mg AA/g), in some subjects are just non-existing, compared with *Longissimus dorsi* m. (23.82 mg AA/g). The histidine, arginine, leucine, lysine, phenylalanine, proline and serine mean in adult sheep made for the two muscle regions showed significant differences (Table 2).

Statistical interpretation of data for amino acid content in *Longissimus dorsi* muscle by age reveals significant differences ($p<0.001$), isoleucine recorded higher averages in lambs (38.07 mg AA/g vs. 35.2 mg AA/g in sheep) in contrast to tryptophan which recorded higher averages values in adult sheep (52.9 mg AA/g vs. 44.27 mg AA/g). The average values of threonine for the same muscle *Longissimus dorsi*, showed significant distinct differences ($p<0.01$) by age also valine and methionine showed significant differences ($p<0.05$).

The amino acids mean values of *Triceps brachii* muscle varied by age, revealing very significant differences ($p<0.001$) for histidine and tryptophan also significant distinct differences ($p<0.01$) for threonine and methionine.

Comparing the essential amino acids average values in lambs by muscular region have been recorded very significant differences ($p<0.001$) for histidine, tryptophan and methionine also significant differences ($p<0.05$) for arginine. *Longissimus dorsi* muscle for both age categories distinguish with higher average values for all essential amino acids parameters excepting tryptophan which recorded higher averages values for *Triceps brachii* muscle in lambs. Thus, variations were noted in mean essential amino acid between the two muscle regions from adult age group, tryptophan revealing very significant differences ($p<0.001$), threonine and methionine showing significant distinct differences ($p<0.01$) also isoleucine and valine revealed significant differences ($p<0.05$).

Analyzing the *Triceps brachii* muscle in terms of non-essential amino acid content according to age, they showed very significant differences ($p<0.001$) for tyrosine, significant distinct differences ($p<0.01$) for alanine and aspartic acid and significant differences ($p<0.05$) for glycine and serine (Table 2). Most of nonessential amino acids parameters from *Triceps brachii* muscle (alanine, aspartic acid, serine, cystine, glutamic acid and proline) had higher mean values in lambs than adult sheep, where higher average values were found only for glycine and tyrosine.

The non-essential amino acids average values recorded of lamb varied by muscle region, revealing very significant differences ($p<0.001$) for tyrosine (21.03 mg AA/g in *Longissimus dorsi* m. vs. 0.73 mg AA/g in *Triceps brachii* m.), significant distinct differences ($p<0.01$) for cystine (13.82 mg AA/g in *Longissimus dorsi* m. vs. 11.9 in *Triceps brachii* m.) and significant differences ($p<0.05$) for glycine (37.4 mg AA/g in *Longissimus dorsi* m. vs. 33.8 mg AA/g in *Triceps brachii* m.).

Analyzing the adult sheep results in terms of non-essential amino acid content according to the muscle region, they have revealed very significant differences ($p<0.001$) for glycine and tyrosine (37.4 mg AA/g *Triceps brachii* m. vs. 31.69 mg AA/g *Longissimus dorsi* m. and 22.2 mg AA/g in *Longissimus dorsi* m. vs. 19.09 mg AA/g in *Triceps brachii* m.), significant distinct differences ($p<0.01$) for alanine and glutamic acid where higher values were recorded in *Longissimus dorsi* m. and for cystine which had higher values in *Triceps brachii* m., significant differences ($p<0.05$) for aspartic acid with higher averages values in *Longissimus dorsi* m.

CONCLUSIONS

Our paper aims to complete the panel information regarding the chemical composition of sheep meat by age in males of Karakul breed.

In the present study, age showed highly significant effects ($p<0.001$) for essential amino acids: isoleucine, tryptophan and histidine and significant distinctly ($p<0.01$) in threonine. Muscle region had very significant effects ($p<0.001$) for histidine and tryptophan and significant (distinctly $p<0.01$) for threonine.

For lambs were obtained superior biological values for both muscles, and in terms of muscle region, *Longissimus dorsi* has remarkable biological.

REFERENCES

[1] Associazione Scientifica di Produzione Animale (ASPA) - Metodologia relativa alla macellazione e dissezione della carcassa degli animali di interesse zootecnico, ISMEA, (1989), Roma.

[2] Bosch L., Alegria A., Farrè R. - Application of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods, Journal of Chromatography *B*, 2006, vol. 831, p. 176-183;

[3] Fengchang W. şi Tanoue E. – Sensitive determination of dissolved tryptophan in freshwater by alkaline hydrolysis and HPLC, 2001, Analytical Sciences, vol. 17.

[4] Friedman M. - Nutritional value of proteins from different food sources, J. Agric. Food Chem., 1996, 44(1): p. 6-29.

[5] Gilka J., Jeliner P., Jankova B., Knesel P., Masek J. şi Docekalova, H. - Amino acid composition of meat, fatty acid composition of fat and content of some chemical elements in the tissue of male lambs fed monensin or lasalocid, Meat Science, 1989, vol. 25, p. 273.

[6] Liu H.J. - Determination of amino acids by precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and high-performance liquid chromatography with ultraviolet detection", Journal of Chromatography A, 1994, vol. 670, p. 59-66.

[7] Loest C.A., Ferreira A.V, Van der Merwe H.J. - Chemical and essential amino acid composition of South African Mutton Merino lamb carcasses, S. Afr. J. Anim. Science, 1997, vol. 27 (1), p. 7-12.

[8] Mancini R. A., Hunt M. C. – Current research in meat colour, 2005, Meat Science, vol. 71(1), p. 100-121.

[9] Sanudo C., Alfonso M., San Julian R., Thorkelsson G., Valdimarsdottir T., Zygoyiannis D., Stamataris C., Piasentier E., Mills C. Berge P., Dransfield E., Nute G. R., Enser M., Fisher A. V. – Regional variation in the hedonic evalution of lamb meat from diverse production systems by consumers in sic European countries, 2006, Meat Science, vol. 75, p. 610-621.

[10] Sheridan R., Hoffman L. C. şi Ferreira A. V. - Meat quality of Boer goat kids and Mutton Merino lambs - Commercial yields and chemical composition, J. of An. Science, 2003, vol. 76, p. 63-71.

[11] Smith R. F. şi Dobson H. – Effect of preslaughter experience on behaviour, plasma cortisol and muscle pH in farmed red deer, 1990, Veterinary record, vol. 126, p. 155-158.

[12] Smith R.H. - Comparative amino acids requirements, 1980, Proc. Nutr. Soc., vol. 39, p.71.

[13] Volpelli L. A., Valusso R., Morgante M., Pittia P. şi Piasentier E., Meat quality in male follow deer (dama dama): effects of age and supplementary feeding, 2003, Meat Science, vol. 65(1), p. 555-562.

[14] Yildiz F., Advances in food biochemistry, 2009, vol. 44(1), p. 6-29.

[15] Waters AccQ.Tag chemistry package - instruction manual, 1993, manual number WAT052874, Millipore Corporation.