

ABSTRACT

At this moment, an increasing worldwide number of producers and processors are concerned with obtaining food of superior quality, under economically profitable conditions. Since food quality, implicitly the level and quality of nutrients from food, is a primordial factor that counts in the assessment of the state of health, it is necessary that the food raw materials producing industry takes into consideration all possible ways of improving the nutritional value of foods. However, data on some qualitative indexes of ovine meat, which could otherwise contribute to a larger picture of the nutritional-biological properties of this product, occurs rather infrequently in the field literature, especially when consumers are interested in meat presently commercialized in Romania.

The main criterion that determined the choice of the doctorate thesis theme was the fact that despite the existence of a wealth of information on the effects of production systems and internal factors on ovine meat quality, these have not yet been compared to those in the NE region of Romania. Considering the existing tradition of ovine breeding in the aforementioned region but also to the inclusion of our country in the European Union, leading to market liberalization, it is considered opportune to carry a study that would serve as a foundation for the characterization of this activity sector and would investigate ovine meat quality from nutritional-biological and sensorial perspectives, in order to improve participation on the European market. Meat quality is one of the elements that can support the renown of ovine breeds from the NE of Romania.

The major aim of the present research consists of the study of elements of blood biochemistry and hematology (red blood cell, white blood cell and blood platelet indexes, total protein concentration, lipid, mineral and energetic profile) with impact upon the metabolic status of ovines with the aim of describing the physiological status and investigating morphostructural attributes of muscle influencing qualitative characteristics of meat production.

The doctorate thesis **“RESEARCH ON THE CORRELATIONS BETWEEN THE MORPHOPHYSIOLOGICAL STATUS BEFORE SLAUGHTER AND NUTRITIONAL-BIOLOGICAL PROPERTIES OF OVINE MEAT”** presents the effects of various factors determining variations of the metabolic state of ovines before reception in slaughterhouses, effects of *Longissimus dorsi* muscle morphostructure from a histological standpoint on meat marbling, maturation and tenderizing, extent of the knowledge on the nutritional value of ovine meat, especially on information regarding intrinsic quality of meat lipids, being of use for

managing a healthy diet for the human organism, biological parameters of meat regarding the essential amino acids content for optimization of protein use in human diets, technological parameters from a qualitative standpoint, the knowledge of these being necessary to processors, in order to provide efficient processing afterwards, and sensorial parameters variations with impact upon meat quality depending on different feeding systems of animals.

The paper has been structured in two distinct parts: the first part, which uses two chapters to present a field literature radiography of the large variety of published results, referring to breeding and physiological factors that can influence meat quality, and the second part of personal research, detailed within eight chapters within which general objectives have been based on assessing effects determined by breed, age at slaughter, gender, season, feed and muscular region on the morphophysiological profile of ovines, which in turn influences the nutritional-biological, physical, technological and sensorial quality of meat. Moreover, within the thesis are also contained the summary, the introduction, conclusions, suggestions and bibliographical sources used.

The plentiful scientific research performed nationally and internationally stressed the practical importance of knowing the animals' natural resistance and capacity of adapting, circumstances of applying advanced ovine feeding technologies, which are influenced by various factors such as: feeding and grazing regime, climatic and environmental conditions, microclimate, transport, therapeutic and immunopreventive activities, prolonged and powerful stress. As a whole, these reduce the animals' adaptation mechanisms, increase their sensibility to pathogenic agents and decrease production quality causing economic loss. Factors such as concentrating a large number of ovines in a space that limits their movement, feeding alimentary additives to animals and growth promoters, failure of ensuring protection of the animals against pathogenic agents and parasites may determine metabolism dysfunctions and alter homeostasis. Physiological, nutritional and pathological condition was assessed by using hematologic and biochemical blood parameters and morphologic parameters from muscle samples. Nutrition, age, gender, reproduction, sheltering, environment, stress and transportation have impact upon hematological, biochemical and morphostructural parameters of animals.

In order to achieve the proposed objectives, investigations were carried on a total number of 104 ovines (52 lambs and 52 adults), respectively 26 lambs of Karakul breed and 26 lambs of Țurcană breed, 26 adult ovines of Țurcană breed (13 males and 13 females) and 26 ovines of Karakul breed (13 males and 13 females), taken from the farm of Horlești, located in the north-east of Romania, in proximity of the Iași municiple.

Taking into consideration that the experimental protocol of the current research contains monitorization of the physiological status and characterization of meat from a physico-chemical, biological, morphological, technological and sensorial standpoint, harvesting and sampling imposed the use of two types of tissue: muscular tissue (*Longissimus dorsi*, *Triceps brachii*,

Deltoideus, *Obliquus abdominis externus*, *Intercostalis*, *Trapezius pars thoracica* and *Trapezius cervicalis*) and blood tissue. These implied setting specific parameters, respectively:

- blood parameters: hematological – red blood cell indicators (red blood cell count – RBC, hemoglobin – HGB, average red blood cell volume – MCV, average red blood cell hemoglobin – MCH, average hemoglobin concentration – MCHC), white blood cell indicators (total white blood cell count – WBC), blood platelet indicators (total blood platelet count – PLT) and biochemical indicators (total protein, albumin, glucose, cholesterol, triglycerides, calcium and magnesium);
- histological parameters determined by cytometric measurements: average myocyte thickness, profile and transversal section surface of muscular fibers, number of fibers composing a muscular fascicle, myocyte/mm² density, striated muscle amount and connective tissue amount composing and defining the *Longissimus dorsi* muscles from a morphological standpoint;
- physical parameters: dynamics of meat acidity in the course of meat maturing, tenderness dynamics (Warner-Bratzler forces) in the course of meat maturing and meat color (L*, a*, b*, C, h⁰);
- chemical parameters: brute chemical composition (dry substance, moisture, total lipids, organic substances, extractive non-nitrous substances, total mineral substances), energetic value, intramuscular lipid profile in saturated and unsaturated fatty acids (essential: ω3 and ω6);
- biological parameters of proteins (essential and non-essential amino acids);
- technological parameters: water holding capacity (losses while boiling);
- sensorial parameters: texture (succulence, toughness, masticability and fibrousness) and aroma (bullion smell, sweet smell, milk smell, mutton smell, acid taste, metallic taste, degree of fat covering in the mouth and persistence of taste).

The analysis methods used in personal research are included in a legislative frame provided in Romanian Standards, in conformity to the European Union legislation and International Standards or to methods used in reference papers.

The assessment of ovine morphophysiological status before slaughter revealed the following aspects:

Hematologic profile indicators assessed on the blood samples from Karakul and Țurcană ovines taken into study highlighted insignificant differences following the statistical analysis comparing young and adults in terms of red blood cell count, hemoglobin, hematocrit, average red blood cell volume, average red blood cell hemoglobin content, white blood cell and blood platelet count, except the average red blood cell hemoglobin content, where significant differences were found in the case of both breeds. Based on the obtained results it can be found that modifications of hematologic status can be correlated to age, characterized by growth of red blood cell count, of hemoglobin concentration, of the hematocrit and of the average red blood cell volume along with aging, results that have been confirmed by other authors from the field literature.

As for the influence of spring after a long time of stabling, reduced values of red blood cell count, hemoglobin, hematocrit, average red blood cell volume, average red blood cell hemoglobin concentration, total proteins and uric acid have been found in ovines from both breeds (Țurcană and Karakul). Thus one can conclude that feed administered during stabling has been quantitatively insufficient, poor in protein substances and unbalanced, favoring animal sickness (occurrence of various lesions in organs or anemia), which imposes optimization of technological management for winter-spring seasons.

The results of our research highlighted the fact that dehydration, fear, or stress are physiological states leading to important changes in hematologic and biochemical variables that form the foundation of assessing the ovines' health state, also serving as an indicator of their welfare. Within the analyzed season (spring) there were encountered variations of all studied hematologic indexes, but the obtained values do not reflect eventual pathological states but only adaptation reactions of the organism to the environment. The decrease of the red blood cell count during spring in studied populations is associated with increase of water consumption and represents the physiological response of the organism in order to maintain its thermal equilibrium in conditions involving higher temperatures. Thermal stress also leads to decrease of ACTH production, which in turn acts by reducing the red blood cell count.

Biochemical profile indicators determined on blood samples from studied ovines of Karakul and Țurcană breeds, following statistical analysis, showed insignificant differences between young and adults for each separate breed, for: total protein concentration, albumin, cholesterol, triglycerides, glucose, amylase, alkaline phosphatase, calcium and aspartate aminotransferase (Țurcană breed) and magnesium for the two breeds.

The reduced level of serum proteins in the spring season was a consequence of a feed deficiency in leguminous hay, which contributed to the decrease of the total serum protein level.

Obtained results within our experiments showed calcium concentration as established within the limits mentioned in the field literature, as a consequence of the use in spring feed of alfalfa hay, which is a rich source of vitamins and mineral substances, especially calcium.

Current research showed that hematologic and biochemical profile values in studied lamb and adult ovine populations were influenced by the cold season and specific feeding during stabling time, but these variations represent animal organism adaptation to environmental conditions, ensuring dynamical balance.

The assessment of histological characters of *Longissimus dorsi* muscles from ovines with different ages

The thickest fibers composing the *Longissimus dorsi* muscles were highlighted in Karakul breed males aged 4 years, with average dimensions of 376,3 μ , and the thinnest fibers have been found particularly in muscle samples taken from 20 days old lambs, respectively 185,7 μ .

Through micrometric measurements performed it is emphasized that the structure of I order muscular fascicles is formed of a higher number of myocytes in young ovines and a lower one in adult males. Thus in lambs, for a muscular fascicle of the order I there are necessary between 18 and 25 fibers and in 4 years old adult males there are 12 myocytes involved. Moreover, myocyte density/mm² presents superior values in fascicles composing lamb muscles (2998 – 3853) than in adult males where myocyte density is much lower (982), a fact that can be explained through the extension of fibers along with ageing and animal growth, hence the diminution of the myocytes/mm² number. Regarding myocyte density per muscular surface unit, it appears to be directly proportional to the number of muscular fibers composing a primary fascicle. Therefore, we can conclude that the *Longissimus dorsi* muscles presenting the finest texture were sampled from lambs aged 25 days old, while muscles sampled from adult ovines presented a coarser texture, fact also found through the resistance opposed to mastication, these results corresponding to those presented by *Priolo and col. (2003)*.

The muscular tissue amount is higher in adult males – 81,31% vs. 79,5% in adult females, the latter presenting a higher amount of connective tissue (20,47% compared to 18,68%) than adult males. Moreover, muscles sampled from adults stood out as richer in connective tissue than those sampled from lambs, results that are correlated to reduced tenderness (measured through Warner Bratzler forces) of meat originating from adult ovines.

The assessment of physical properties of ovine meat

The evolution of the pH during ovine meat refrigeration/maturation is characterized by oscillatory amplitude of recorded values, following a descending trend during the first 48 h, then an ascending trend within the time span of 48-120 h, when meat maturation is stimulated.

At the end of the rigidity period it is found that in muscle samples taken from the Karakul breed, the highest glycogen values are found in the *Longissimus dorsi*, as for Țurcană they are found in *Trapezius pars thoracica*. pH values during meat maturation fit within qualitative standards for meat, avoiding undesired effects such as PSE or DFD.

Varying age or weight at slaughter is one of the most important factors for ovine meat acidity, during the *prerigor mortis* phase. Resulted statistical differences in pH values between the two age categories for muscle samples from the two breeds taken into study (Karakul and Țurcană) are justified by the level of energetic resources present in muscles due to age, with higher glycogen quantities in adult ovines.

Warner Bratzler shearing forces values presented a descending tendency during meat maturation, deduced from measurements executed at 6, 14 and 120 h postmortem.

The highest tenderness values were recorded in the fifth day of meat maturation at the temperature of 2° C, being associated with protein degradation taking place during refrigeration, and the lowest values were recorded at 6 h after slaughter, results confirmed by several authors in the field literature. Warner Bratzler shearing forces values were higher in muscle samples

from adult ovines than in those taken from young ovines, explainable through the increase of connective tissue resistance along with ageing.

Through the comparison between the average values of Warner Bratzler shearing forces obtained for muscle samples from lambs and those recorded at adult ovines, we can assert that *Longissimus dorsi* muscles from young ovines are the tenderest during maturation stage.

Moreover, it can be found that muscle samples from lambs are more tender than those from adult carcasses, resulting into highly significant differences for all the values of Warner Bratzler forces measured during biochemical modifications taking place in muscles, after slaughter.

Through global colorimetric characterization of ovine meat it has been found that the influence and specific print is composed by the fat content, age and feeding regime, over the L*, a*, b*, C, h indexes, especially over L*, a*, b*. Therefore:

- muscle samples from the ovine young ovine carcasses presented superior luminosity compared to homologous muscles from adult carcasses, situation confirmed by other authors;
- muscles from adult ovine carcasses showed superior values of the “a” coordinate (red-green) compared to those from lambs, since myoglobin concentration increases with ageing;
- for the “b” coordinate (yellow-blue) superior values have been recorded in samples originating from adult ovines, being associated with higher intramuscular fat content, due to the feeding regime different from the young ovines’, the yellow color resulting from high beta-carotene levels contained by the green feed introduced in adult ration.

The assessment of the chemical composition of ovine meat

Based on the chemical components constituting ovine meat, the *Longissimus dorsi* and *Triceps brachii* are considered to be qualitatively superior, under the aspect of nutritional-dietetic value (high protein content, reduced calorificity and low fat content).

By analyzing the dry substance content in each studied muscular region reported to age, it is found that obtained values for samples from male adult ovines are superior to those originating from young ovines of both breeds, and reported to gender, it is found that muscles sampled from adult males present a higher average dry substance content compared to those from adult females.

Average values of protein amount show composition uniformity in the analyzed seven muscle groups, with maximal values recorded preponderantly in samples corresponding to adult females. The *Longissimus dorsi* muscles stand out as having the highest protein content within analyzed samples from both breeds. By analyzing protein content as related to age, for the Karakul breed, it is found that muscles from adult ovines presented superior average values compared to those from lambs, with the exception of *Trapezius pars thoracica*.

Lipids in ovine meat constituted the component with the highest oscillatory amplitude from analyzed muscular regions, average resulted values fitting within the variation range with the low limit of 2,77 (*Longissimus dorsi* from lambs of Karakul breed) and the high limit of

17,22 (*Trapezius cervicalis* from adult males of Țurcană breed). Muscle samples from ovine carcasses presented in its constitution lipids with superior percentages in *Trapezius cervicalis*, *Obliquus abdominis externus* and *Trapezius pars thoracica*.

Calculated energetic value showed the highest calorificity values in cervical and abdominal muscles from adult ovine carcasses (both females and males), due to the muscle category as well as to age. By analyzing from the perspective of the “gender” factor, it is found that muscles originating from males present superior values to those corresponding to females. Overall, it is found that the *Longissimus dorsi*, *Triceps brachii* and *Intercostalis* are the most dietetic muscles from young ovine carcasses.

The nutritional-biological value of ovine meat is reflected by its essential amino acids content, being a primary premise for maintaining a normal balance of nitrous bases in the organism. Muscles from ovine carcasses presented within their composition proteins with a structure from which essential amino acids such as tryptophan, leucine, lysine, arginine and valine stood out in the *Longissimus dorsi* and *Triceps brachii*. The protein constitution in essential amino acids specific to *Longissimus dorsi* sourced in young ovines presented superior values corresponding to each amino acid, excepting tryptophan with upper limits corresponding to samples from adult ovines.

By comparing average values recorded for essential amino acids for the two muscle categories from adult ovine carcasses it is found that the *Longissimus dorsi* represent the extreme superior values in oscillatory amplitude for all essential amino acids, compared to *Triceps brachii*.

Statistical analysis indicated that slaughtering ovines at different age categories presents highly significant influences on isoleucine, tryptophan and histidine, and distinctly significant on threonine. By statistically comparing recorded values for essential amino acids for the two muscular regions there was found the existence of very significant differences for histidine and tryptophan and distinctly significant for threonine. The meat originating from the two muscles in the young category has superior biological value, from analyzed muscles the *Longissimus dorsi* stand out as superior.

The results of performed research indicate that ovine slaughter at different age categories does not modify only the content in saturated, monounsaturated and polyunsaturated fatty acids but also the $\omega 6:\omega 3$ ratio in the *Longissimus dorsi* and *Triceps brachii*. Thus, it has been found that as age increases, the SFA in *Longissimus dorsi* remains relatively constant, the PUFA content decreases along with the increase of MUFA, while *Triceps brachii* presented a decrease of SFA, increase of PUFA content and decreased MUFA content. The decreased of the SFA amount in the *Triceps brachii* may be associated with the decrease of serum cholesterol found in blood, which leads to decreased risk of cardio-vascular diseases. During accumulation of intramuscular fat deposits, it has been found that SFA and MUFA present higher increase in comparison to PUFA, presenting repercussions on the PUFA content decrease and automatically

over the SFA/PUFA ratio. These differences are recorded as under the influence of genetic and feeding factors in animals, fact noticeable through the large variation range of values obtained from the analysis of fatty acids composing intramuscular lipids. The PUFA/SFA ratio as well as the PUFA ω -6/ ω -3 ratio represent two important parameters in the assessment of lipids in meat, ovine muscle samples being characterized by a PUFA/SFA ratio varying between 0,4 and 0,68, and the PUFA ω -6/ ω -3 ratio presented values ranged between 1,91 and 3,43. Results of actual research detailing the lipid profile in fatty acids describe a meat rich in PUFA, average obtained values being comparable to those from ovines that have been fed with PUFA-rich feed. Despite the general perception that most of the animal fats would be constituted by SFA, approximately 60% of the fatty acids from meat of studied ovines are unsaturated.

Research results indicate that *Longissimus dorsi* are qualitatively superior to *Triceps brachii* due to their lower SFA content (39,66% in *Longissimus dorsi* vs. 45,57% in *Triceps brachii*), the latter being possibly correlated to an increased fat level in meat, leading to the development of a large cholesterol circulating in lipoproteins. We have found a decrease of the SFA percentage and the increase of the PUFA percentage, due to the abundance of linolenic acid (C18:3 ω 3) in the green feed representing the nutritive base, so this acid is deposited in significant amounts in their tissues. Ovine meat presents a high percentage of ω 3 unsaturated fatty acids, thus recommending it for consumption, for its positive impact over the reduction of the incidence of cardio-vascular diseases (*Longerie, 1994*). The PUFA:SFA ratio presents values fitting within the range of 0,12 – 0,54. Considering that the literature (*Department of Health Guidelines, 1994*) recommends values of the PUFA/SFA of 0,45%, it can be argued that the ovine species provides food that can cover the necessities of a balanced diet.

The technological assessment of ovine meat highlighted that muscle samples from young ovine carcasses expressed higher boiling losses than those from adult ovines in both analyzed groups of muscles (*Longissimus dorsi* and *Triceps brachii*). By comparing resulted losses through boiling for the two muscle groups, it was found that *Longissimus dorsi* presented superior values compared to *Triceps brachii* in both studied age categories.

Sensorial analysis of ovine meat quality based on the feeding regime highlighted existing highly significant differences by applying the Fisher analysis test between the three meat batches originating from lambs fed only milk, lambs fed milk and feed supplements and lambs fed milk and green feed for the following characters: initial succulence, succulence persistence, toughness, masticability, fibrousness, milk smell, mutton smell, acid taste, metallic taste and taste persistence. By applying the Tukey statistical test, pairs between which these differences occurred were highlighted.

Meat originating from lambs fed milk and green feed is considered to be less tender, more difficult to masticate and more fibrous than the meat from lambs fed milk and feed supplements. Observed variability between the different types of meat can be explained through



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differences regarding age and the physical effort performed by lambs from analyzed groups, which can generate confusions related to the used feed. Meat originating from lambs fed exclusively milk stood out as having the highest succulence in comparison to corresponding characters identified in the other two meat batches.

By analyzing the aromatic profile it has been found that animals fed milk and green feed produced meat with strong mutton flavor, livery smell and a much more persistent taste and meat originating from animals fed exclusively milk presented more perceptible metallic/sanguine characters, sweet smell and milk smell.

Following the complete nutritional-biological value resulted from research, ovines of the Țurcană and Karakul breeds are recommended for breeding and reproduction for obtaining lambs and crossbreeds to be fattened and capitalized upon.