SUMMARY

of the doctoral thesis entitled:

STUDY ON THE PHYSICO-CHEMICAL AND MORPHOLOGICAL CHARACTERISTICS OF MEAT DEPENDING ON ORIGIN AND PRESERVATION METHODS

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The meat from economically significant species is a primary component in human nutrition, which is why it is constantly under the scrutiny of researchers.

The aim of this research was to monitor the physico-chemical and morphological changes that can occur in meat, depending on the method of obtaining it, namely the slaughter of animals in conventional, halal or traditional systems. Therefore, the present study aims to highlight, through the measurement of cortisol and other biochemical stress enzymes in serum, such as catalase (CAT), superoxide dismutase (SOD) and serum malondialdehyde (TBARS), in complementarity with pH determination and histological examination of muscle samples, the changes that occur in meat depending on the breeding system and the slaughtering method practiced, in short, based on its origin.

Considering that there is a lack of well-documented information about animal stress during slaughter, its measurement and the impact on meat quality, the main objective was to review animal stress at the time of slaughter, stress management and its impact on meat quality.

The research conducted in this study took place in eight slaughter units and in household, and laboratory analyses were carried out at the Faculty of Veterinary Medicine in Bucharest, specifically in the Food Control Laboratory, the Biochemistry Laboratory and the Pathological Anatomy Laboratory. Additionally, some determinations were performed in an external laboratory.

The doctoral thesis entitled "Study on the Physico-Chemical and Morphological Characteristics of Meat Depending on Origin and Preservation Methods" is structured

into two main parts (literature review and original research), in accordance with current legal provisions.

Section I, the Bibliographic Study, spans 50 pages. This section is structured into five chapters and presents general information about the physico-chemical characteristics of meat and the changes that can occur in meat after animal slaughter. Additionally, it emphasizes the influence of animal stress on meat, depending on their origin.

Section II, Personal Research, comprises 105 pages and is structured into six chapters: the Purpose and Objectives of the Research, Materials and Methods, Results of the Comparative Study on the Variation of Biochemical, Physico-Chemical and Morphological Parameters Based on the Origin of Pigs, Cattle, Sheep and Poultry, as well as the final chapter of General Conclusions and Recommendations.

Thesis contains a total of 44 tables and 131 figures. At the end, there are five Anexes, spanning a total of 63 pages.

Section I: Bibliographic Study

Chapter I. Study on the Physico-Chemical Characteristics of Meat from Economically Significant Animals provides information about the macroscopic and microscopic structure of muscle tissue.

Chapter II. Physico-Chemical and Morphological Changes in Meat Obtained after Animal Slaughter discusses the fact that after animal slaughter, a series of biochemical transformation occur in the muscles and describes the factors that influence them.

Chapter III. The Influence of Stress on Slaughtered Animals on the Physico-Chemical and Morphological Characteristics of Meat describes how the animal organism reacts to pre-slaughter stress and defines some of factors that can increase their stress levels. Additionally, the last subsection details a series of methods through which we can reduce stress in animals during slaughter.

Chapter IV. Methods of Obtaining Meat Depending on Origin highlights theoretical aspects related to breeding systems, as well as the slaughter methods applied to economically significant animals (cattle, sheep, pigs, poultry). It describes stunning methods practiced in conventional slaughter, as well as the aspects to consider in traditional, halal or kosher slaughter, thus respecting religious precepts.

Chapter V. Study on Meat Consumption and Human Health highlights a series of healthy dietary recommendations and describes studies where consumers assess meat quality.

Section II: Personal Research

The aim and objectives summarize the importance of the current research.

Chapter VI. Materials and Methods comprises three subsections with information that structures and details the institutional framework in which the

research was conducted, the materials used, and the working methods applied in the research.

The study included a total of 198 mammals and birds, of which 65 were pigs, 51 cattle, 45 sheep, 26 broilers and 11 turkeys. Blood and muscle samples collected from these animals were processed at the Faculty of Veterinary Medicine in Bucharest, specifically in the Food Control Laboratory, the Biochemistry Laboratory, and the Pathological Anatomy Laboratory. Additionally, cortisol determination was carried out in an external laboratory. For each animal, additional information such as species, breed, sex, age, body weight, breeding system and slaughter system were recorded.

In the following chapters (VII, VIII, IX, X), the quantification of cortisol from slaughtered animals through various methods is pursued (conventional, halal, traditional), according to species (pigs, cattle, sheep, poultry), along with conducting comparative studies of the obtained results. In each chapter, the determination of stress enzymes through biochemical methods is also presented, as well as tracking the physico-chemical changes that may occur in meat, depending on the method of obtaining it, namely the slaughter of animals (conventional system, halal system, traditional system) and depending on the method of refrigeration (cooling at 2-4°C). Histological analysis of muscle samples collected from animals slaughtered in slaughterhouses or household and determining how the breeding system and slaughter method influence the morphological characteristics of meat are presented for each species under study.

Chapter VII. Comparative Study on the Variation of Biochemical, Physico-Chemical, and Morphological Parameters, Depending on the Origin of Pigs, presents how the method of breeding and slaughter influences the level of cortisol, catalase, SOD, TBARS, glucose and total proteins in serum samples.

Blood samples collected from pigs were divided into three groups: a group of pigs slaughtered conventionally in June, a group of pigs slaughtered conventionally in November, and a group of pigs slaughtered traditionally. As a result, the highest values of cortisol levels were recorded in the group slaughtered conventionally in November, compared to the group slaughtered conventionally in June, which is correlated with the temperatures to which the pigs were exposed. Temperature is an important factor that influences the level of stress in animals. Additionally, samples collected from pigs raised in household and slaughtered traditionally recorded lower cortisol levels compared to samples collected from conventionally slaughtered animals. This is likely correlated with the animals' rearing conditions, as they do not experience the stress of transportation and are not kept in crowded batches.

All data were statistically processed using SPSS v.26 software. Initially, the samples were analyzed for normal/anormal distribution using the Shapiro-Wilk test. Subsequently One-Way ANOVA Test was applied, revealing significant differences in cortisol levels depending on the slaughter method applied to the pigs.

Furthermore, statistically, it was found that the slaughter method applied to pigs significantly influences the levels of SOD and TBARS, with lower values observed in pigs slaughtered in the traditional system.

To understand how the breeding method and slaughter method influence meat quality, pH determinations were made, as well as histological examinations of muscle samples. It was faund that the passage of time influences the pH value of pork meat, with statistically significant differences observed between pH values measured on meat samples stored by refrigeration at 2-4°C for six days.

Regarding the histological examination of muscle samples collected from conventionally and traditionally slaughtered pigs, differences in the amount of adipose tissue were observed between two groups, most likely due to the feeding regimen received by the animals on the farm versus in household.

Chapter VIII. Comparative Study on the Variation of Biochemical, Physico-Chemical, and Morphological Parameters, Depending on the Origin of Cattle, presents how the breeding method and slaughter method influence the animals' stress level and hence the meat quality, through the quantification of cortisol and catalase in serum samples collected from bovines during the time of slaughter. To understand how the breeding method and slaughter method influence meat quality, pH determinations were made, as well as histological examinations of muscle samples.

The samples were divided in four groups: cattle slaughtered conventionally in the cold season, cattle slaughtered conventionally in the warm season, cattle slaughtered halal and cattle slaughtered traditionally. It was found that all stages of the technological flow of cattle slaughter are respected in the studied slaughterhouses, both for conventionally slaughtered cattle and for those slaughtered halal and traditionally.

As a result, regarding the level of measured cortisol, samples collected from conventionally slaughtered cattle obtained a lower medium value compared to the medium value obtained for serum samples collected from traditionally and halal slaughtered cattle. This is likely correlated with the stress of transportation (the cattle from the four groups were slaughtered in the slaughterhouse, requiring transportation from the farm or household where they were raised to the slaughtering facility), individual reactivity of each animal, and the season inwhich the bovines were slaughtered.

As in the previous chapter, all data obtained were statistically processed using SPSS v.26 software. Initially, the samples were analyzed for normal/anormal distribution using the test Shapiro-Wilk, and subsequently, the non-parametric Kruskal-Wallis test was applied. The analysis revealed that the slaughter method influences the cortisol level, with significant differences observed between the conventional 1 - halal, conventional 1 - traditional, conventional 2 - halal and conventional 2 - traditional groups.

Regarding the catalase value, the results of the Mann-Whitney test showed that the slaughter method does not influence its level, as there were no statistically

significant differences between the groups of halal and conventionally slaughtered cattle.

The influence of time passing, and thus the deterioration of meat quality, affects the pH value, with statistically significant differences observed, analyzed through the Kruskal-Wallis statistical test.

Following the histological examination of muscle samples collected from the thigh area of conventionally and halal slaughtered cattle, predominantly normal muscle tissue with low representation of adipose tissue was observed. Transportation stress and the presence of parasitic formations such as *Sarcocystis spp.* may be causes of muscle fiber degeneration, with interstitial reaction frequently observed.

Chapter IX. Comparative Study on the Variation of Biochemical, Physico-Chemical, and Morphological Parameters, Depending on the Origin of Sheep, presents how the breeding method and slaughter method influence the stress level of sheep and hence the meat quality, through the quantification of cortisol and catalase in serum samples collected at the time of sheep slaughter, as well as through pH determination and histological examination of muscle samples.

The samples were divided into three groups: conventionally slaughtered sheep, halal slaughtered sheep and traditionally slaughtered sheep.

Samples collected from lambs slaughtered traditionally in household, obtained lower average cortisol values compared to the medium values of samples collected from lambs slaughtered halal and conventional. This is likely correlated with the rearing method, the absence of transportation stress and the fact that lambs are not keept in crowded batches.

All data obtained were statistically processed using SPSS v.26 software. Applying the Kruskal-Wallis test resulted in finding that the slaughter method influences the cortisol value, with statistically significantly differences observed between the traditional - halal and traditional - conventional groups.

Following the statistical analysis of the three groups of sheep, applying the One-Way ANOVA test, it resulted that the slaughter method influences the catalase values.

The passage of time influences the pH value of lamb meat, with statistical significantly differences ($p < 0.05$) observed between its values measured at certain time intervals.

The histologically examined images showed a normal aspect of muscle fibers, both in cross-section and longitudinal section. The muscle samples, preserved by refrigeration at 2-4°C for 24 hours, exhibited rigid muscle fibers with a characteristic wavy appearance.

Chapter X. Comparative Study on the Variation of Biochemical, Physico-Chemical, and Morphological Parameters, Depending on the Origin of Poultry, presents how the breeding method and slaughter method influence the stress level of poultry and hence the meat quality, through the quantification of cortisol in serum and the determination of pH in muscle samples.

The samples from broilers were divide into 3 groups: conventionally slaughtered broilers, halal slaughtered broilers and traditionally slaughtered broilers. Additionally, a separate group was formed with samples collected from turkeys.

Within the studied slaughterhouses, the technological steps of slaughtering poultry are observed, with the conventional method being used for broilers, involving electric stunning and gas stunning being practiced for turkeys.

All samples collected from poultry (broilers and turkeys) obtained a result of <1 µg/dL, likely correlated with their short lifespan and low body weight.

The passage of time influences the pH value of poultry meat, with significant differences statistically observed between its values measured at certain time intervals.

The histological examination of the samples showed muscle fibers with a normal appearance alternating with discrete sheets of adipose tissue, especially in broilers raised in household, most likely due to the diet they received.

The number of samples collected from poultry was not extended because the results obtained from cortisol quantification were inconclusive.

Considering that turkeys are slaughtered at a more advanced age and have a higher body weight compared to broilers, cortisol quantification was attempted for them as well. However, the results were identical to those obtained for broilers. Therefore, further investigations with other groups slaughtered by different methods were not extended.

Chapter XI. General Conclusions and Recommendations comprise all the conclusions and recommendations formulated based on the research of this doctoral thesis.

The ***Bibliography*** includes a total of 252 bibliographic sources consulted

The thesis concludes with a list of publications as a first author, corresponding author, or co-author. During the doctoral period, I have published a total of 30 scientific papers, including 7 full papers in ISI-indexed journals with impact factor, 12 full papers in ISI-indexed (ESCI) journals, and 11 full papers in BDI-indexed journals. Specifically related to the thesis topic, 4 papers have been published, including 3 indexed in ISI (ESCI) journals and one indexed in BDI journals. Additionally, I have participated in various conferences with 34 papers presented orally or as posters.