PhD THESIS SUMMARY

The quality and hygiene of beef and poultry meat obtained within some production chains

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INTRODUCTION

The approach of this doctoral thesis had the main purpose of bringing more up-to-date scientific information, regarding meat from the main species of meat-producing animals in Romania, and in the case of our study, we considered meat from cattle and poultry.The importance of beef and poultry consumption is given by the specific sensory characteristics, nutritional composition, economic-technological aspect and food safety.

Regarding the quality of beef, we know that it can be influenced by several factors, among which we mention the breed of the animals. There are significant variations in the physico-chemical composition of beef, such as fat, protein and mineral content, due to different breeds of cattle. The assessment of the impact of breed on the physico-chemical composition is important for the quality of the final food intended for the consumer. This assessment plays an important role in optimizing the selection of beef cattle breeds.

The evaluation of the microbiological contamination of cattle carcasses in the main stages of slaughter is an important stage for food safety.

The evaluation stages of cattle pathologies in the main authorized slaughterhouses are an important aspect to maintain a high standard of food safety. Identifying diseases and conditions that can influence animal health before the slaughter stage is essential to prevent meat contamination. The importance of the characterization of these pathologies helps to establish protocols of sanitary-veterinary hygiene and control of existing diseases.

One of the most important species supplying meat from us in the country is represented by the bird, so the evaluation of the prevalence of E. Coli. in poultry meat is important. Escherichia coli is one of the most common causes of food poisoning related to the consumption of poultry meat.

Regarding the prevalence of E. coli in poultry meat, it represents an important indicator of hygiene and food safety measures for the entire processing chain. The work has an impact on the final product but also on the consumer's confidence in the food sector.

THESIS STRUCTURE

The doctoral thesis entitled " The quality and hygiene of beef and poultry meat obtained within some production chains" comprises 157 pages and presents 11 tables and 89 representative figures for each separate chapter.
The first part of the thesis - The Current State of Knowledge, consists of 51 pages and is structured in 3 chapters.

Chapter I - "General considerations regarding meat quality", includes general information from specialized literature, the concept of quality, characteristics and influencing factors. We also scored the evaluation of the commercial quality of meat-producing animals.

Chapter II - titled "Evaluation of meat quality from a physico-chemical and microbiological point of view", includes the main notions regarding the physico-chemical composition of meat, the biochemical transformations that take place in meat as well as other biochemical processes that occur in meat.

Chapter III - "Assessments through risk analysis", presents general notions regarding the HACCP system, the stages of risk analysis and the potential risks regarding meat production.

The second part of the thesis - Personal contribution. It comprises a number of 105 pages and is structured in 6 important chapters. Regarding the content of this part, it refers to information and data obtained throughout the entire doctoral study. The information refers to the working hypothesis regarding the proposed experiment, the purpose and main objectives pursued within each study, the samples and the methods used. Also in this part, the results obtained in each study are presented, and they are highlighted by correlation with existing data in the specialized literature.

We evaluated the quality of beef composition according to breed, namely the microbiological contamination of cattle carcasses in the main stages of slaughter, the prevalence of pathologies in cattle in authorized slaughtering units and finally we also evaluated the prevalence of E. Coli in chicken meat.

The end of this PhD thesis concludes with a special chapter of general conclusions and one of information regarding the originality of this research study.

RESEARCH OBJECTIVES

1. Evaluation of the impact of breed on the compositional quality of beef.

2. Analysis of the microbiological contamination of cattle carcasses in the main stages of slaughter.

3. Evaluation and characterization of pathologies in cattle in units authorized for slaughter in Romania.

4. Analysis of the prevalence of E. coli in poultry meat.

RESULTS AND DISCUSSION

Chapter IV- Study 1-„Evaluation of the compositional quality of beef according to breed'', in this chapter we followed the differences in physical-chemical composition, depending on the breed. The main objective was to obtain percentage values regarding the results of the beef from the three considered breeds (Băltătă Românească, Belgian Blue and Black Angus) and compared with results from the specialized literature. The compositional parameters that were evaluated in the study are represented by the amount of water, protein and fat. The FoodScan analyzer was used. Protein values were lower in the Belgian Blue followed by Black Angus. The water content varied between 75.93% for Balță Românească and 76.29% for Black Angus. The lowest amount of fat was in the case of the Romanian Balţata breed, followed by Black Angus.

The main conclusion indicates that breed can be a main influencing factor, regarding the values of physico-chemical composition of beef. This study attests that the parameters of the physico-chemical composition in the two considered slaughterhouses show average individual values very close to the reference.

Chapter V - Study 2 - "Evaluation of the microbiological contamination of cattle carcasses in the main stages of slaughter" in which we aimed to evaluate the prevalence and microbiological risk factors associated with beef.

This study proposed a risk analysis regarding the potential biological hazards in the two essential stages of slaughtering, namely, evisceration and the cooling or blasting stage.

The dangers evaluated were both the main hygiene parameters, evaluated normally, and the potential pathogens possibly existing at the level of the intestinal flora and then possibly transmitted by non-observance of strict hygiene rules.

Regarding the main objectives pursued, in this study, were the following: the determination of the total number of germs (NTG m.a) and the total number of Enterobacteriaceae from the carcasses during the cooling/thrusting stages, at the level of two slaughterhouses of different capacities; assessment of the possible presence of pathogenic bacteria, such as Salmonella spp., pathogenic Escherichia coli and Listeria spp. at the level of intestinal microflora; evaluation of the possible presence of potentially pathogenic bacteria (E. coli, Salmonella spp., Listeria spp.) during the cooling/thawring stages); confirmation by molecular methods and rapid automated methods of the pathogenicity of the main bacteria isolated within the studied chain, respectively the analysis of the severity and probability of microbiological risk in the production chain and in the units studied based on the results obtained and their evaluation from a statistical point of view.

Regarding the results regarding the hygiene parameters analyzed from the carcasses (NTG m.a.; Enterobacteriaceae), the evaluation of the number of aerobic colonies (NTG m.a.) in the cattle slaughterhouses in Romania, we identified significant variations between the samples taken immediately after evisceration and those collected during the cooling stage, so that the post-evisceration samples indicated NTG values between 2.1 and 2.8 log CFU/cm^2, while samples from the cooling area showed statistically significantly lower values ($P < 0.05$), between 1.1 and 1.9 log CFU/cm^2.

The mean value of NTG after evisceration was 2.2 log CFU/cm^2, considerably higher than the mean of 1.5 log CFU/cm^2 recorded during the cooling phase, underlining the importance of carcass hygiene controls during the processing stages. The results of Enterobacteriaceae levels also showed clear differences between these stages, with 84% of samples from the cooling area (n = 42) recording values below 1.0 log CFU/cm^2. In contrast, post-evisceration counts ranged between 0.7 and 2.2 log CFU/cm^2, with three samples exceeding 2.0 log CFU/cm^2. The mean value for Enterobacteriaceae in the cooling zone was 1.7 log CFU/cm^2, lower than the mean of 1.9 log CFU/cm^2 recorded after evisceration. Thus, the significant differences between post-evisceration and chilling underline the effectiveness of the chilling process in reducing bacterial load in this study.

Regarding the isolation of E. coli, of the total samples analyzed (n = 50), 14% (n = 7) were confirmed as pathogenic E. coli. Regarding the presence of Salmonella spp. in faecal samples, 14 positive samples were found, representing 28% (n = 50). As well as the presence of Listeria spp. bacteria, our study revealed the presence of a large number of positive samples, but none confirmed pathogenic by PCR for the specific sequences of Listeria monocytogenes.

The main conclusions drawn from the study are the following:

1. The significant differences between the total microbial load (TMB) post-evisceration (average: 2.2 log CFU/cm^2) and after the cooling stage (average: 1.5 log CFU/cm^2) underline the effectiveness of the hygiene measures implemented during processing.

2. Higher levels of Enterobacteriaceae post-evisceration (mean: 1.9 log CFU/cm^2) compared to those in the cooling stage (mean: below 1.0 log CFU/cm^2 in 84% of samples) indicate that evisceration is a vulnerable stage for contamination in both slaughterhouses studied.

3. The significant reduction in Enterobacteriaceae load in the cooling stage demonstrates the effectiveness of temperature control and ventilation methods in limiting bacterial proliferation.

4. Monitoring of NTG and Enterobacteriaceae is essential to assess the hygienic quality of meat processing.

5. High initial contamination followed by a significant reduction indicates that rigorous processing can reduce the risk of microbial contamination to acceptable levels.

6. The experiment reveals a 14% prevalence of E. coli STEC in the analyzed faecal samples, with the dominant serogroup O101. Therefore this aspect underlines the regional specificity of the predominant serogroups and the relevance of their monitoring.

7. The presence of serogroup O26, although rarer, remains significant with important public health implications given its association with severe foodborne illness in Europe.

8. The presence of Salmonella spp. in feces, with a prevalence of 28%, and the identification of S. typhimurium and S. enteritidis species highlights a significant risk of contamination during processing.

9. The lack of Listeria monocytogenes in the faecal and carcass samples reflects a favorable situation for the two studied slaughterhouses, but the prevalence of Listeria spp. in two faecal samples suggests the need for continuous monitoring.

10. The significant reduction in bacterial load (E. coli, Salmonella) between the post-evisceration stage and the cooling phase underlines the importance of hygiene control during processing.

11. Differences in pathogen prevalence compared to other regions and studies suggest that processing methods, cattle husbandry practices and analytical methods significantly influence results.

We therefore support the implementation of food safety policies that include strict preventive measures and rapid identification of pathogenic serogroups, especially in traditional growing and processing systems.

Chapter VI – Study 3 - "Evaluation of the prevalence of pathologies in cattle in authorized slaughter units" this study had the following main objectives:

- Evaluation of the prevalence of ante-mortem lesions in cattle in authorized slaughterhouses;
- Identification and classification of macro-pathological lesions, post-mortem in cattle;
- Carrying out the histopathological examination of post-mortem lesions;
- Biological risk analysis and food safety risk assessment;

Regarding the results of the ante-mortem inspection performed on cattle, it indicates a low incidence of ante-mortem injuries, within the study. From the total of 110 cattle examined, six specimens showed obvious changes in their health status, which corresponds to a percentage of 5.45%. Each type of change was observed only once, representing an individual weight of 0.90%.

Following the post-mortem inspection, it was found that, of the 110 cattle examined, 60 had post-mortem lesions, which represents 54.54%.

From the analysis of the data on the prevalence of certain lesions, it was observed that most of the lesions were located in the lungs (31 lesions), followed by those in the liver (20 lesions), kidney (12 lesions), oral cavity (9 lesions), lymph nodes (8 lesions), the musculoskeletal and joint system (7 lesions) and the mammary glands (1 lesion). The percentage distribution of the lesions is as follows: 28.18% of the lesions were located at the lung level, 18.18% at the liver level, 10.90% at the kidney level, 8.18% at the oral cavity level, 7.27% at the lymph nodes, 6.36% at the musculoskeletal and joint level and 0.90% at the breast level.

Regarding liver and kidney damage, they also represent a significant percentage, which can be related to possible metabolic or toxic conditions. Oral cavity and lymph node lesions suggest localized infection or inflammation, while musculoskeletal and joint lesions may be associated with trauma or inadequate care. Breast lesions, although less common, indicate possible problems with the hygiene and health of the mammary glands.

At the respiratory level, we encountered the following injuries: lobar pneumonia (0.90%); fibrinous pneumonia with adhesions (1.81%); necrotic pneumonia (2.72%) and suppurative bronchopneumonia (3.63%). Emphysema observed in this study were: alveolar pulmonary emphysema (5.45%) and represents the most common lesion identified, interstitial emphysema (2.72%) and bullous emphysema (0.90%).

Other lesions we observed: bronchiectasis (3.63%), pulmonary fibrosis (1.81%), pulmonary hemorrhage (1.81%), and mineralized pulmonary cysts (0.90%), which are rare lesions and could indicate isolated cases of chronic infections or calcifications associated with previous diseases. It should be noted that the granulomatous lesions that are specific to tuberculosis were absent in this study.

Following the post-mortem examination of cattle, it was observed that the most common liver lesion was hepatic telangiectasia with a prevalence of 8.18%, followed by hepatic steatosis (2.72%), hepatic abscess (1.81%), hepatic fibrosis (1.81%) and granulomatous hepatitis (1.81%). Other lesions observed with lower prevalence were cholangiohepatitis and hepatic congestion, both of which had a prevalence of 0.90% in our study.

Analyzing the data obtained, following our study, we found that renal congestion was the most frequent lesion, with a prevalence of 4.54%, followed by interstitial nephritis (possibly associated with White spotted disease) and chronic interstitial nephritis with multiple cysts, both with a prevalence of 0.90%. Other lesions identified were: renal hemorrhage (0.90%), renal infarction (0.90%), nephritis with renal stones (0.90%), renal fibrosis (0.90%) and multifocal interstitial nephritis (0.90%). Next, we found that lingual ulcer was the most common lesion with a prevalence of 5.45%, followed by anodontia (1.81%) and viral papilloma (0.90%).

Subcutaneous hemorrhages are the most common lesions observed, indicating mechanical trauma suffered by the animals before or during slaughter. Causes may include improper handling, inadequate transportation, or failure to observe good cattle handling practices. These lesions not only affect carcass quality, but also raise animal welfare concerns. Myositis, necrosis and muscle gangrene, although less common, are signs of poor hygiene or poor animal care. The lack of bursitis and arthritis associated with brucellosis in the study is a positive indicator regarding the sanitary-veterinary status of the examined cattle.

The most important conclusions drawn in our study are the following:

1. In both studied abattoirs, a low incidence of ante-mortem injuries was revealed, only 5.45% of the cattle examined had ante-mortem injuries, indicating a relatively good general state of health.

2. No significant differences were recorded between the analyzed slaughterhouses.

3. The types of lesions identified (horn avulsion, dyspnea, necrotic dermatitis) suggest deficiencies in compliance with hygiene and animal welfare norms.

4. A high prevalence of post-mortem injuries was found.

5. Approximately 28.18% of the post-mortem lesions were located at the lung level.

6. A high frequency of liver telangiectasia lesions and renal congestion was found.

7. The lesions identified suggest repeated exposure to stressors and pathogens.

8. No granulomatous lesions specific to bovine tuberculosis were identified in this study.

9. Low prevalence of musculoskeletal injuries (6.36%), suggests that mechanical trauma is less problematic compared to respiratory and metabolic infections.

Chapter VII – Study 4 - "Evaluation of the prevalence of Escherichia Coli in poultry meat".

The current study was conducted to analyze the prevalence of Escherichia coli (E. coli) in poultry meat samples, as well as to assess the antimicrobial susceptibility of these isolates.

Following the isolation protocol, a total of 30 E. coli isolates were recovered from the 100 samples analyzed (30/100; 30% prevalence). Almost all isolates recovered in our study showed resistance phenotypes (96.66%).

The results indicate that all recovered E. coli were susceptible to AMK, IPM, MEM, ETP and AZT. Most isolates were found to be resistant to TET (24/30; 80%), AMP (24/30; 80%), SMX (22/30; 73.33%), CHL (21/30; 70%) and NA (18/30; 60%).

Strong resistance to CIP (17/30; 56.66%), TMP (15/30; 50%), CTX (14/30; 46.66%), CAZ (13/30; 43.33%) and GEN (12/30; 40%) was also observed. E. coli isolates showed low percentages of resistance to FEP (7/30; 23.33%), FOX (6/30; 20%) and CST (1/30; 3.33%). Furthermore, more than 70% of the isolates were found to be multidrug resistant, exhibiting resistance to at least three different classes of antibiotics.

Consistent with their resistance phenotypes, antimicrobial resistance determinants detected among E. coli isolates included tetA (16/30; 53.33%), tetB (14/100; 46.66%), blaTEM (11/30; 36.66%), sul1 (8/100; 76.66%), aad. 23.33%), blaCTX (5/30; 16.66%), blaOXA (5/30; 16.66%), qnrA (5/30; 16.66%) and aac (3/30; 10%).

The most important conclusions drawn from this experiment are the following:

1. The study analyzed the prevalence of E. coli in poultry meat samples and evaluated the antimicrobial susceptibility of the recovered isolates. Moreover, the presence of several AMR genes was also detected.

2. E. coli was identified in 30% of the samples collected.

3. The study highlights the role of poultry meat as a reservoir of AMR E. coli, emphasizing the importance of continuous monitoring of the spread of AMR in the food chain.

4. Assessing the prevalence and AMR of E. coli is very important, both for food safety reasons and for analyzing the impact on public health and the spread of AMR bacteria in humans.

GENERAL CONCLUSIONS

1. The parameters of the physico-chemical composition of the beef from the two studied slaughterhouses show average individual values very close to the reference specific to each individual component, thus we can state that the meat samples collected from all three breeds of cattle correspond in terms of composition.
2. The significant differences between the total microbial load (TMB) post-evisceration (average: 2.2 log CFU/cm^2) and after the cooling stage (average: 1.5 log CFU/cm^2) underline the effectiveness of the hygiene measures implemented during processing.
3. Higher levels of Enterobacteriaceae post-evisceration (mean: 1.9 log CFU/cm^2) compared to those in the cooling stage (mean: below 1.0 log CFU/cm^2 in 84% of samples) indicate that evisceration is a vulnerable stage for contamination in both slaughterhouses studied.
4. The significant reduction in Enterobacteriaceae load in the cooling stage demonstrates the effectiveness of temperature control and ventilation methods in limiting bacterial proliferation.
5. High initial contamination followed by a significant reduction indicates that rigorous processing can reduce the risk of microbial contamination to acceptable levels.
6. 14% prevalence of E. coli STEC in the analyzed faecal samples, with the dominant serogroup O101. This emphasizes the regional specificity of the predominant serogroups and the relevance of their monitoring adapted to the local context.
7. The presence of serogroup O26, although rarer, remains significant with important public health implications given its association with severe foodborne illness in Europe.
8. The presence of Salmonella spp. in feces, with a prevalence of 28%, and the identification of S. typhimurium and S. enteritidis species highlights a significant risk of contamination during processing.
9. The lack of Listeria monocytogenes in faecal and carcass samples reflects a favorable situation for the two studied slaughterhouses, but the prevalence of Listeria spp. in two faecal samples suggests the need for continuous monitoring.
10. The significant reduction in bacterial load (E. coli, Salmonella) between the post-evisceration stage and the cooling phase underlines the importance of hygiene control during processing.
11. The results of the study emphasize the need to improve the monitoring of zoonotic pathogens in Romania, with an emphasis on E. coli STEC and Salmonella.
12. A low incidence of ante-mortem lesions was revealed, only 5.45% of the cattle examined had ante-mortem lesions, indicating a relatively good general state of health.

13. No significant differences were recorded between the abattoirs analyzed, suggesting an even distribution of ante-mortem injuries and similar animal management in all locations.
14. The types of lesions identified (horn avulsion, dyspnoea, necrotic dermatitis) suggest deficiencies in compliance with hygiene and animal welfare norms.
15. A high prevalence of post-mortem lesions was found, with 54.54% of the cattle examined showing post-mortem lesions, a significant percentage compared to the European average, which varies between 15-30%.
16. About 28.18% of the post-mortem lesions were located at the lung level, suggesting a high prevalence of respiratory diseases.
17. A high frequency of liver telangiectasia lesions and renal congestion was found.
18. No granulomatous lesions specific to bovine tuberculosis were identified.
19. Low prevalence of musculoskeletal injuries (6.36%).
20. E. coli was identified in 30% of the samples collected. E. coli isolates were recovered in the current study and substantial resistance to several classes of antibiotics was shown, which is concerning.
21. Our study highlights the role of chicken meat as a reservoir of AMR E. coli, emphasizing the importance of continuous monitoring of the spread of AMR in the food chain.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The originality of this thesis can be seen in its integrated approach from several key points of research, which make important contributions to the field of food safety and microbiological monitoring. The evaluation of the parameters of the physico-chemical composition of beef from the two studied slaughterhouses aligns with the reference values, indicating that they comply with the composition standards.

The research provides an image of the risks of contamination and the effectiveness of hygiene implementation measures, respectively the assessment of the microbial load in certain stages and the monitoring of the prevalence of E. coli spp, Salmonella spp. and Listeria spp. Another important aspect is the evaluation of injuries in slaughterhouses in Romania, especially in the context where the prevalence of post-mortem injuries is much higher than the European average.

From the point of view of contributions at the national level, we can mention the compositional comparative evaluation of the main beef producing breeds in Romania; identification of the prevalence of pathogenic strains of E. coli, characterized molecularly, attesting to regional specificity and the relevance of their monitoring; the identification and confirmation of the main pathogenic strains that indicate the need to improve the monitoring of zoonotic pathogens in Romania, with a predominant focus on E. coli STEC, Salmonella spp. and Listeria monocytogenes, respectively the attestation of the prevalence of E. coli, in poultry meat as well as the evaluation of the susceptibility of pathogenic strains to antimicrobials, being a novelty for Romania.

Contributions at the international level stand out through the development of knowledge in the field through ISI and BDI publications, regarding compositional quality, relevant pathogens in slaughterhouses, the incidence and prevalence of E. Coli in carcasses. The results highlight original aspects and offer possibilities for their transformation in medical and socio-economic practice.

This doctoral thesis highlights detailed scientific aspects from the point of view of the physical-chemical, microbiological and pathological examinations carried out, having a remarkable contribution to the field of meat quality and hygiene, being an integrated model of applied research.