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MICROBIOLOGICAL AND HYGIENIC QUALITY OF AUBRAC CATTLE FRESH MEAT

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Abstract

This work aimed to investigate and evaluate the microbiological control of carcasses from Aubrac cattle, focusing on ensuring quality and food safety in the meat industry. In this research, three microbiological parameters were monitored: contamination with Salmonella, total bacterial count and contamination with Enterobacteriaceae. The sponge sampling method was employed, involving the wiping of a 100 cm^2 surface area with a template using sponges soaked in sterile diluted peptone. Sampling was carried out randomly from ten carcasses (males and females), selecting four areas with the highest contamination frequency from each carcass, resulting in a total surface area of 400 mm^2. Following the tests, it was seen that the samples did not contain any bacteria from the Salmonella genus or the Enterobacteriaceae family. In terms of the total number of bacteria, the highest microbial load was found in males (8.2 x 102 cfu/cm^2) on carcasses 2 and 5. Simultaneously, the lowest microbial load (6.0 x 102 cfu/cm^2) was recorded on carcass 4 from females. We can conclude that, based on the results, these values are below the accepted standard limit, indicating wholesome meat.

Key words*: beef carcass, food safety, microbiology.*

INTRODUCTION

The Aubrac breed is a cattle breed originating from the Aubrac region in southern France. It is known for its resilience and adaptability to environmental conditions, adapting very well to the pedo-climatic conditions in Romania (Madescu et al., 2021). The meat from Aubrac cattle is appreciated for its superior quality, with a fine texture and delicate flavor. It is considered high-quality meat due to its tenderness and high marbling content, which contribute to its juiciness and flavor (Bakharev et al., 2017).

The quality of food products has a much broader significance than that of other products, as it has much deeper effects since it is fundamental to life, determines the course of metabolic processes, and can influence the development of the entire organism. Microbial contamination of carcasses occurs as a result of inadequate hygiene conditions during slaughter, processing, and meat handling. Contamination can be reduced through good processing practices, but the complete elimination of pathogenic germs is nearly impossible. Numerous techniques have been developed to reduce contaminating bacteria on carcass surfaces, but most current procedures involve washing and disinfection.

The meat intended for consumption must come from healthy animals, as some diseases can be transmitted to humans through ingesting contaminated pieces (Clinquart et al., 2022). Due to its chemical composition, especially its highwater content, meat provides a conducive environment for the growth of microorganisms. Risks associated with consuming fresh meat include infections caused by certain bacteria, such as Salmonella, Lister, and Enterobacteriaceae, as well as the presence of parasites (Atsbha et al., 2018).

Microorganisms are extremely small, making it impossible to observe them with the naked eye during post-mortem inspection. However, visual inspection of the meat can detect lesions, fecal contamination, and various foreign bodies that may constitute contamination (Tesson et

al., 2020). To specifically identify microorganisms in meat, specific laboratory techniques are required (Fegan et al., 2004).
In any Hazard Analysis and Critical Control Point (HACCP) program for primary meat processing, the condition of the animal represents a critical control point. The physiological state of the animal and the internal and external microbial load are important factors for the final microbiological quality of the meat after slaughter (Manyori et al., 2017). This does not mean that other factors, such as facility design, slaughter procedures, and adherence to good practice standards, are not important in ensuring the production of hygienic and high-quality carcasses.
Generally, it is assumed that preventing visible contamination of carcasses will enhance the microbiological safety of the meat (Warmate et al., 2023).Visible carcass contamination can be reduced by washing, skinning before flaying, and paying increased attention to evisceration and flaying so as not to transfer dirt to the meat (Gonzales-Barrón et al., 2016).It would be preferable to control visible contamination through superior flaying and evisceration practices rather than by applying washing treatments that may not always promote the removal of a substantial number of bacteria from carcasses. Washing reduces visible contamination and does not affect the microbiological condition of the carcass, but in the worst case, due to humidity, bacteria can multiply more easily (Brown et al., 2013).
Determining the microbiological parameters of Aubrac beef has multiple benefits. This information can be used to assess and improve quality, monitor production and hygiene processes, ensure food safety, and meet consumer requirements and preferences. Additionally, these evaluations can contribute to promoting and valorizing high-quality meat and developing a more sustainable food industry (Cummins et al., 2016).

MATERIALS AND METHODS

For the purpose of this research, the detection of bacteria from the genus Salmonella, the total bacteria count, and contamination with Enterobacteriaceae on carcasses originating from Aubrac cattle were pursued.

Sampling from carcasses is carried out according to the SR ISO 17604/2009 standard, utilizing both destructive and non-destructive methods (Figure 1).
In this research, the sponge swabbing method was employed, where a surface area of 100 cm^2 was wiped using the template using sponges soaked in sterile peptone diluent. The wiping procedure was repeated 10 times vertically and 10 times horizontally at the selected site.

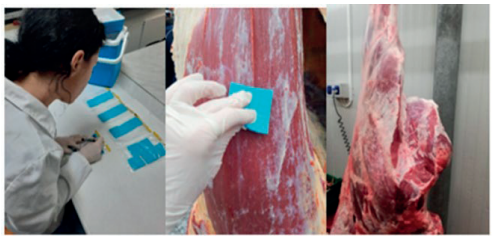


Figure 1. Sampling from carcasses (original foto)

The sampling was conducted randomly from five carcasses, selecting four areas from each carcass with the highest contamination frequency, resulting in a total surface area of 400 mm^2.After sampling, all samples were packaged and labelled, ensuring they were sealed. The next step involved sending the samples to a specialized laboratory under appropriate conditions to avoid any alterations to their quality at the time of collection.
After sampling, to detect bacteria from the Salmonella genus, the horizontal method according to standard SR EN ISO 6579-1:2017/A1:2020 was used. For conducting the analysis, the following materials and glassware were used: sterile Petri dishes of various sizes, graduated or automated pipettes, sterile Pasteur pipettes or sterile pipettes, a pH meter, test tubes or bottles, a water bath, and apparatus for dry sterilization (oven) or wet sterilization (autoclave).
For preparing culture media and reagents, the following components were used: non-selective pre-enrichment medium: buffered peptone water (BPW); selective enrichment media: Rappaport-Vassiliadis soy broth (RVS broth) and Muller-Kauffman-tetrathionate-novobiocin broth (MKTTn broth); selective solid isolation media: xylose lysine deoxycholate agar (XLD agar); and a second medium of choice.

To make sure the biochemistry was correct, different types of agar (Christensen), physiological saline solution, medium for lysine decarboxylation, and agglutination serums for somatic "O" and "H" antigens were used.
Initially, a 25 g sample was taken for analysis and inoculated with 225 ml of buffered peptone water. The sample was then incubated at a temperature of 37°C ± 1°C for 18 hours ± 2 hours.
In the selective enrichment stage, 0.1 ml of the previously obtained culture was transferred to a test tube containing 10 ml of RVS broth. Additionally, 1 ml of culture was transferred to a test tube containing 10 ml of MKTTn broth. The RVS broth was incubated for 24 hours ± 3 hours at a temperature of 41.5°C ± 1°C, while the MKTTn broth was incubated for the same period of time but at a temperature of 37°C ± 1°C.
After the selective enrichment stage, the first selective medium (XLD agar) was inoculated using a loop with the culture obtained from the RVS broth. Similarly, the second selective medium was inoculated using the culture from the MKTTn broth. The two media were incubated for 24 hours at a temperature of 37°C ± 1°C, and the results were to be examined.
For confirmation, at least one suspicious colony was selected from each plate, and if the first colony was negative, another four colonies were taken. These colonies were streaked on nutrient agar plates and incubated at 37°C ± 1°C for 24 hours (Figure 2).

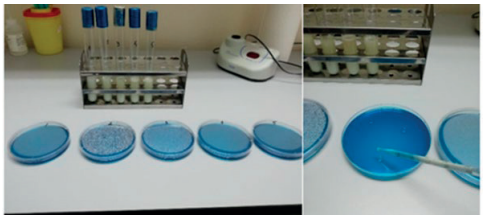


Figure 2. Plate streaking
(original photo)

For determining the total germ count, the horizontal method for enumerating microorganisms at 30°C was used, in accordance with standard SR EN ISO 4833-1/2014. This method involves the use of the following materials and equipment: dry or wet sterilization apparatus, incubator, Petri dishes, pipettes, water bath, colony counting device, and test tubes. Ten milliliters of the sample to be analyzed were mixed with ninety milliliters of physiological saline, which is the horizontal method for counting microorganisms. For inoculation and incubation, one plate was used for each dilution, transferring 1 milliliter of sample using new sterile pipettes for each plate. Subsequently, approximately 12-15 milliliters of PCA culture medium at a temperature of 44-47°C were poured into each Petri dish. The time between preparing the initial suspension or dilutions and pouring the medium into plates did not exceed 45 minutes. The inoculum was carefully mixed with the medium by rotating the Petri dishes, and then the mixture was allowed to solidify with the plates placed on a cool horizontal surface.
After complete solidification of the mixture, the plates were inverted and incubated at a temperature of 30°C ± 1°C for 72 ± 3 hours. At the end of the incubation period, the colonies on the plates were counted using a special device equipped with magnifying lenses, which facilitates colony counting (Figure 3).



Figure 3. Colony Counting
(original photo)

The horizontal method for detecting and enumerating Enterobacteriaceae was determined using standard SR ISO 21528-2/2017. This utilizes the following culture media: buffered peptone water, VRBG agar, glucose agar, brilliant green bile glucose broth, and oxidase reagent. For conducting the test, sterile Petri dishes, sterile test tubes, graduated pipettes, Pasteur pipettes, inoculating loops, and a test tube rack were required.
Non-selective pre-enrichment was carried out by adding 10 ml of buffered peptone water to the sanitation buffer with which the sample was collected. The mixture was homogenized and left to incubate for 18 hours ± 2 hours at 37°C.
For selective enrichment, 1 ml of the obtained culture was transferred to a test tube containing 10 ml of brilliant green bile glucose broth and

incubated at 37°C for 24 hours ± 2 hours. For colony isolation, an inoculating loop was used to streak the surface of VRBG agar, which had been previously poured into a Petri dish (Figure 4). The plate was then incubated at 37°C for approximately 24 hours.

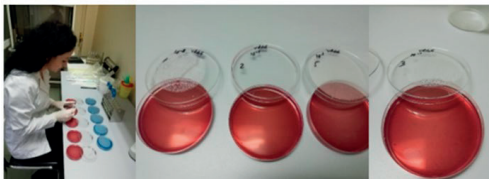


Figure 4. Isolation of Colonies (original photo)

After the results were read, the number of colony-forming units per square centimeter (CFU/cm²) was calculated using the formula: CFU/cm² = N * F * A * D, where N represents the number of colony-forming units per milliliter of dilution liquid, F is the quantity of dilution liquid in the test tube, A is the investigated surface area, and D is the reciprocal of the dilution factor used.

For confirmation, isolated colonies underwent tests for glucose fermentation and oxidase. Thus, colonies showing positive glucose fermentation and a negative oxidase test were identified as Enterobacteriaceae.

After conducting all analyses, the results obtained were read, interpreted, and subsequently centralized.

RESULTS AND DISCUSSIONS

Results regarding Salmonella contamination

After sample collection, the analysis method described in standard SR EN ISO 6579-1:2017/A1:2020 was used to determine *Salmonella* contamination. From the data presented in Table 1, it is observed that the presence of bacteria from the *Salmonella* genus is not detected in the analyzed samples. Both in standards and in specialized literature, it is specified that *Salmonella* must be absent in the entire sample mass under analysis. Thus, we can conclude that the collected samples originate from healthy carcasses.

Table 1. Results regarding the contamination of bovine carcasses with *Salmonella*

Carcass identification number	Gender		Analysis method
	Male	Female	
1	Absent		
2	Absent		SR EN ISO
3	Absent		6579-1:2017/
4	Absent		A1:2020
5	Absent		

Results regarding the total bacterial count

Samples were collected from various areas of bovine carcasses, following standard SR EN ISO 4833-1/2014, and the obtained results are structured in Table 2. For the determination of the total germ count, dilutions of up to 10^2 were used for better interpretation of the results. Subsequently, the read values were multiplied by 10^2. From the obtained result (Table 2), it can be observed that the highest microbial load is found in males (8.2 x 10^2 CFU/cm^2) on carcasses No. 2 and 5. At the same time, the lowest microbial load (6.0 x 10^2 CFU/cm^2) was recorded on carcass No. 4 from females. Thus, the values for the total germ count range between 6.0 x 10^2 CFU/cm^2 and 8.2 x 10^2 CFU/cm^2, and based on the results, we can appreciate that these values are below the standard's permissible limit, resulting in healthy meat.

Table 2. Results regarding the total bacterial count

Carcass identification number	Gender		Analysis method
	Male	Female	
1	6.4 x 10^2 CFU/cm^2	8.0 x 10^2 CFU/cm^2	
2	8.2 x 10^2 CFU/cm^2	6.1 x 10^2 CFU/cm^2	
3	7.3 x 10^2 CFU/cm^2	7.4 x 10^2 CFU/cm^2	SR EN ISO
4	6.4 x 10^2 CFU/cm^2	6.0 x 10^2 CFU/cm^2	4833-1/2014
5	8.2 x 10^2 CFU/cm^2	6.2 x 10^2 CFU/cm^2	
Daily logarithmic mean	**2.86 log CFU/cm²**	**2.82 log CFU/cm²**	

Results regarding contamination with Enterobacteriaceae

Similar to the previous examination, carcasses from both males and females were analyzed (SR EN ISO 21528-2/2007). From the obtained results, it can be observed in Table 3 that the presence of bacteria from the Enterobacteriaceae genus was not detected in the analyzed samples. If no presence of Enterobacteriaceae is detected in a microbiological test of beef, this indicates that organisms from the Enterobacteriaceae family were not identified in the tested sample. This result can be considered favorable in terms of food safety, as the absence of Enterobacteriaceae suggests a low or nonexistent level of bacterial contamination in the tested beef.

Table 3. Results regarding the contamination of carcasses with Enterobacteriaceae

Carcass identification number	Gender		Analysis method
	Male	Female	
1	Absent		SR EN ISO 21528-2/2007
2	Absent		
3	Absent		
4	Absent		
5	Absent		

The absence of Enterobacteriaceae is generally an indicator that hygiene and quality control processes have been effective in preventing bacterial contamination.

CONCLUSIONS

In conclusion, the results of the microbiological analyses indicate a high level of hygiene and food safety in the production and handling processes of beef. The absence of *Salmonella*, total bacterial count, and Enterobacteriaceae bacteria in the analyzed samples demonstrates that hygiene standards have been strictly adhered to at all stages of the process, from slaughter to packaging. These positive results are essential to ensuring consumers that the final product is safe and suitable for consumption. Furthermore, these findings underscore the effectiveness of hygiene control measures and good manufacturing practices applied in the beef industry. As food safety is a major concern for consumers, these results validate the food industry's commitment to providing superior-quality and safe products for consumption.

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*SR EN ISO 21528-2/2007 - Determination of Enterobacteriaceae bacterial family.