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EVALUATION OF RABBIT MEAT MICROBIOTA FROM THE VIEWPOINT OF MARKETING METHOD

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ABSTRACT

Microbiological analysis was performed on carcasses of rabbits coming from domestic slaughter, purchased at butcher shops, vacuum-packaged and purchased in supermarkets, as well as frozen. The total number of analysed rabbits was 20. For all samples the following microbiological parameters were determined: total microorganisms count (TAC), the count of lactic acid bacteria (LAB), psychrotropic microorganisms, moulds and yeasts, as well as bacteria of the Enterobacteriaceae family. Total microorganisms count was the highest (p <0.05) in rabbit meat from butcher shops (5.34 log CFU.g^{-1}). The counts of lactic acid bacteria (LAB) in rabbit meat originating from domestic breeding was 2.58 log CFU.g^{-1}, in vacuum-packaged rabbits 3.18 log CFU.g^{-1}, in frozen rabbits 2.29 log CFU.g^{-1}, and in rabbit meat purchased from butcher shops 3.58 log CFU.g^{-1}. The highest count (p <0.05) of Enterobacteriaceae was observed in samples from butcher shops, namely 2.91 log CFU.g^{-1}. In contrast the lowest count (p <0.05) was in rabbit meat from home slaughtering at 1.47 log CFU.g^{-1} and in frozen ones at 1.36 log CFU.g^{-1}. The lowest counts (p <0.05) of moulds and yeasts were observed in rabbit meat from domestic slaughter, namely 1.12 log CFU.g^{-1}. The highest counts (p <0.05) were in rabbit meat from butcher shops 2.97 log CFU.g^{-1}. The highest counts (p <0.05) of psychrotrophic microorganisms were detected in rabbit meat from butcher shops, namely 4.98 log CFU g^{-1} and the lowest ones (p <0.05) in the meat of domestically slaughtered rabbits at 2.52 log CFU.g^{-1}. In all monitored microbiological indicators, we have found differences (p <0.05) in their counts on the surface and inside the muscle tissue, both on the front and rear parts of the rabbit carcass.

Keywords: rabbit; TAC; LAB; *Enterobacteriaceae*; psychrotrophic microorganisms; **y**easts and moulds

INTRODUCTION

Safety and shelf life of meat is limited by microbial growth. Dominant organisms causing spoilage in carcasses of rabbits and packaged rabbit meat include Gram-negative bacteria, psychrotrophic bacteria, lactic acid bacteria, yeasts, and Brochothrix thermosphacta **(Pereira and Ferreira, 2015)**. In aerobic conditions, most spoilage involves the genus Pseudomonas. However, in vacuum or in modified atmosphere its growth is suppressed **(Corry, 2007; Kameník and Chomát, 2013)**. According to **Rodriguez-Calleja et al. (2004)**, the limiting factor for the shelf life of meat, is the count of microorganisms at 6 to 7 log CFU.g^{-1}. **Pereira and Ferreira (2015)** reported the count to be higher, at 7.00 to 8.00 log CFU.cm^{-2}.

Microbiological quality of rabbit meat can be affected by various factors, such as storage conditions and hygiene during the slaughtering **(Koutsoumanis and Sofos, 2004)**. During the slaughtering, contamination of muscle tissue may be caused by a wide variety of microorganisms, particularly during evisceration, due to an increase in the count of microorganisms originating from the gastrointestinal tract **(Nakyinsigea et al., 2015)**. Nutrition also has a significant influence on the number of microorganisms, as some feed ingredients may adversely affect the rate of growth of microorganisms **(Hernandez, 2008)** and can extend the shelf life of rabbit meat **(Vannini et al., 2003)**.

The objective of the work was to evaluate microbiota of rabbit meat and determine whether marketing method. (packaging, storage) and a sampling site (surface, inside, front, rear parts of the rabbit carcass) have impact on the number of some microorganisms.

MATERIAL AND METHODOLOGY

Microbiological analysis was carried out on samples of rabbit meat derived from domestic slaughter (1 day after slaughtering), rabbit meat purchased from the butcher (3 days before Best before date), vacuum-packaged rabbit meat purchased in supermarkets (3 days before Best before date), and frozen rabbit meat (3 month before Best before date). The total number of analysed rabbits was 20. The samples were transported to the microbiological lab in a thermal bag at 4 °C to avoid violating the refrigeration regimen. The microbiological analysis was carried out in a microbiological lab of the Department of Food Technology at Mendel University in Brno.

Samples were taken from four locations of a rabbit carcass:

- the surface of the front part (part above the last thoracic vertebra, including the front legs),
- inside the muscle tissue of the front part,
- the surface of the rear part (the part below the seventh lumbar vertebra, including the hind legs),
- inside the muscle tissue of the rear part.

For all samples, was determined the following microbiological parameters:

- Total microorganisms count (TAC) – 72 hours at 30 °C **(ISO 4833-1, 2014)**.
- Lactic acid bacteria (LAB) – 72 hours at 30 °C **(ISO 13721, 1998)**.
- Psychrotrophic microorganisms – 10 days at 6.5 °C **(ISO 17410, 2003)**.
- Moulds and yeasts – 5 days at 25 °C **(ISO 21527-1, 2009)**.
- *Enterobacteriaceae* family – 24 hours at 37 °C **(ISO 21528-2, 2006)**.

Sampling and processing was carried out based on **ISO 7218 (2007)** and **ISO 6887-1 (2003)**. All analyses were carried out during the shelf life of the given product.

The following methods were used for statistical evaluation: the calculation of basic statistical parameters (mean, standard deviation, standard deviation of the mean) and the simple sorting method of analysis of variance (ANOVA, Tukey's test). Evaluation was performed using the STATISTICA CZ programme, version 10.

RESULTS AND DISCUSSION

Microbiological quality of rabbit meat in terms of marketing method.

Total count of microorganisms (Figure 1)

The total microorganisms count was the highest (p <0.05) in rabbit meat from butcher shops. Among the samples of vacuum-packaged rabbit meat, frozen meat, and home-slaughtered meat, there was no observed statistical difference (p <0.05) in TAC.

The highest count of microorganisms detected in rabbit meat coming from butcher shops indicates either a failure of the refrigeration regimen during storage, or false data about the shelf life. TAC best describes the degree of microbial contamination of given food and we can guess by this the adherence to technology in the production, transport, and storage **(Görner and Valík, 2004)**. The total microorganisms count in rabbit meat originating from domestic breeding was 3.17 log CFU.g^{-1}, in vacuum-packaged rabbit meat 3.97 log CFU.g^{-1}, in frozen rabbit meat 3.82 log CFU.g^{-1} and in rabbit meat purchased from butcher shops 5.34 log CFU.g^{-1}. **Rodriguez-Calleja et al. (2006)** reported that the total count of bacteria in the rabbit meat usually ranges from 4.01 to 4.96 log CFU.g^{-1}. **Nakyinsigea et al. (2015)** and **Lan et al. (2016)** reported a higher TAC, immediately after slaughter at 4.7, respectively 4.6 log CFU.g^{-1}. After three days of storage, the TAC increased to 6.18 log CFU.g^{-1}, after five days to 6.78 log CFU.g^{-1}, and after seven days the microbial counts reached 7.83 log CFU.g^{-1}. In contrast, lower counts found by **Pereira and Ferreira (2015)**, ranged from 2.87 to 4.87 log CFU.g^{-1}1.

The maximum permitted TAC value in rabbit meat intended for heat treatment is not currently regulated by the Czech legislation. The no longer valid Decree 132/2004 Sb. on the Microbiological Requirements for Foods, their Monitoring and Evaluation stated the TAC limit to be 106 CFU.g^{-1} (6 log CFU.g^{-1}). All our analysed samples complied with this requirement.

Lactic acid bacteria (Figure 2)

The counts of lactic acid bacteria were the highest (p <0.05) in rabbit meat originating from butcher shops and in vacuum-packaged rabbits. The counts of lactic acid bacteria in rabbit meat originating from domestic breeding were 2.58 log CFU.g^{-}1 in vacuum-packaged rabbit meat at 3.18 log CFU.g^{-1}, in frozen rabbit meat at 2.29 log CFU.g^{-1} and in rabbit meat purchased from butcher shops at 3.58 log CFU.g^{-1}.

Lactic acid bacteria are the main organisms causing spoilage of rabbit meat in vacuum packaging **(Rodriguez-Calleja et al., 2010)**, which corresponds with our results. We have detected a higher count of LAB in rabbit meat from butcher shops, which could be due to a prolonged storage. **Pereira and Ferreira (2015)** have also reported similar results for rabbit meat before packaging as they detected 2.21 to 3.71 log CFU.g^{-1} of LAB.

Enterobacteriaceae (Figure 3)

The highest count (p <0.05) of Enterobacteriaceae was recorded in samples of rabbit meat from butcher shops at 2.91 log CFU.g^{-1} The lowest count (p <0.05) was in meat of home-slaughtered rabbits at 1.47 log CFU.g^{-1} and in frozen rabbits at 1.36 log CFU.g^{-1}. **Pereira and Ferreira (2015)** reported similar counts to ours, where before packaging rabbit meat they detected an average of 1.18 log CFU.g^{-1} (<1.00 to 3.27 log CFU.g^{-1}) of Enterobacteriaceae. **Rodriguez-Calleja et al. (2005)**, observed higher counts after seven days of storage and recorded 2.80 log CFU.g^{-1} of Enterobacteriaceae. This result corresponds to the count of Enterobacteriaceae identified in this experiment in rabbit meat coming from butcher shops. The reason why Enterobacteriaceae counts were higher may be due to poor hygienic handling of rabbit meat. This may have occurred during refrigeration, transport or packaging as the rabbit carcasses were packaged into plastic bags later at butcher shops. Poor sanitation of workers may have played certain role **(Steinhauser et al., 2000)**, as Enterobacteriaceae are an indicator of improperly performed hygiene during the manufacturing process and during storage **(Görner and Valík, 2004)**.

Moulds and yeasts (Figure 4)

It was observed the lowest counts (p <0.05) of moulds and yeasts in rabbit meat originating from domestic slaughter at 1.12 log CFU.g^{-1}, the highest (p <0.05) in the rabbit meat from butcher shops at 2.97 log CFU.g^{-1}. Since moulds and yeasts are capable of growing even under very unfavourable conditions, they are some of the common originators of food spoilage **(Vlková et al., 2009)**. In contrast this results, **Pereira and Ferreira (2015)** have found higher counts of yeasts and moulds at 3.92 log CFU.g^{-1} (<1.00 to 3.92), **Chabela et al. (1999)**

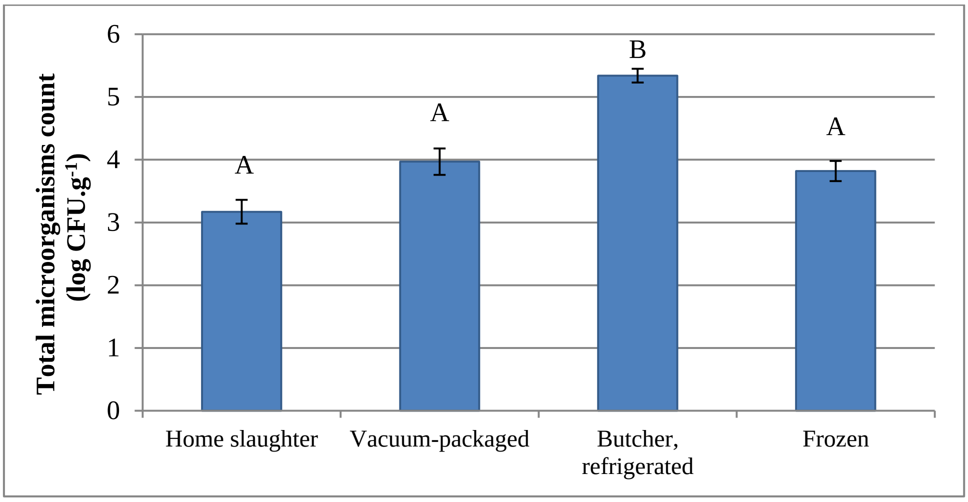

Figure 1 Comparison of the total count of microorganisms (log CFU.g^{-1}) in rabbit meat, (n = 5). Averages marked with different letters in the monitored factor (marketing method) are statistically different (*p* <0.05).

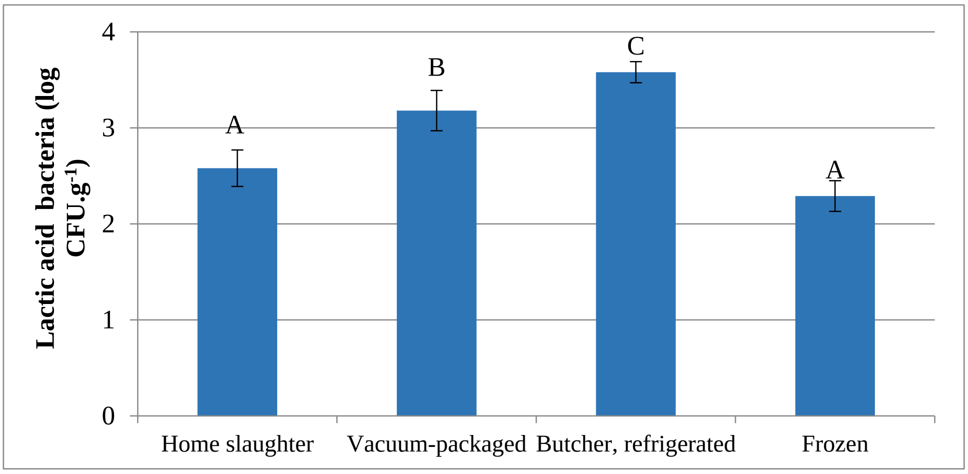

Figure 2 Comparison of the counts of lactic acid bacteria (log CFU.g^{-1}) in rabbit meat, (n = 5). Averages marked with different letters in the monitored factor (marketing method) are statistically different (*p* <0.05).

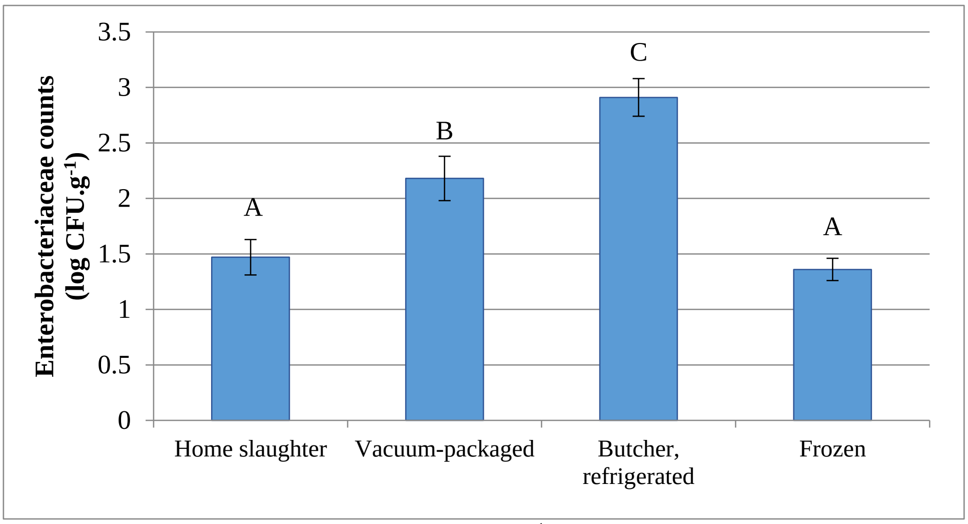

Figure 3 Comparison of *Enterobacteriaceae* counts (log CFU.g^{-1}) in rabbit meat (n = 5). Averages marked with different letters in the monitored factor (marketing method) are statistically different (*p* <0.05).

found 3.76 log CFU.g^{-1}, but microbial analysis was carried out during a total of 14 days of storage.

Psychrotrophic microorganisms (Figure 5)

Figure 5 shows that the highest count (*p* <0.05) of psychrotrophic microorganisms was detected in rabbit meat originating from butcher shops at 4.98 log CFU.g^{-1} and the lowest (*p* <0.05) in the meat from domestic slaughter of rabbits at 2.52 log CFU.g^{-1}. **Pereira and Ferreira (2015)** have detected similar counts of psychrotrophic microorganisms between 2.46 and 5.25 log CFU.g^{-1}. After four days of storage their count, in

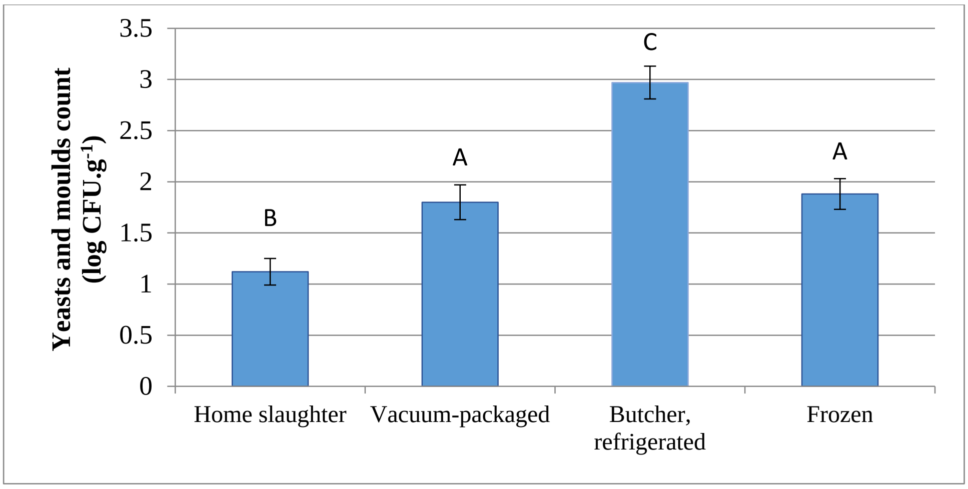

Figure 4 Count comparison of yeasts and moulds (log CFU.g^{-1}) in rabbit meat (n = 5). Averages marked with different letters in the monitored factor (marketing method) are statistically different (*p* <0.05).

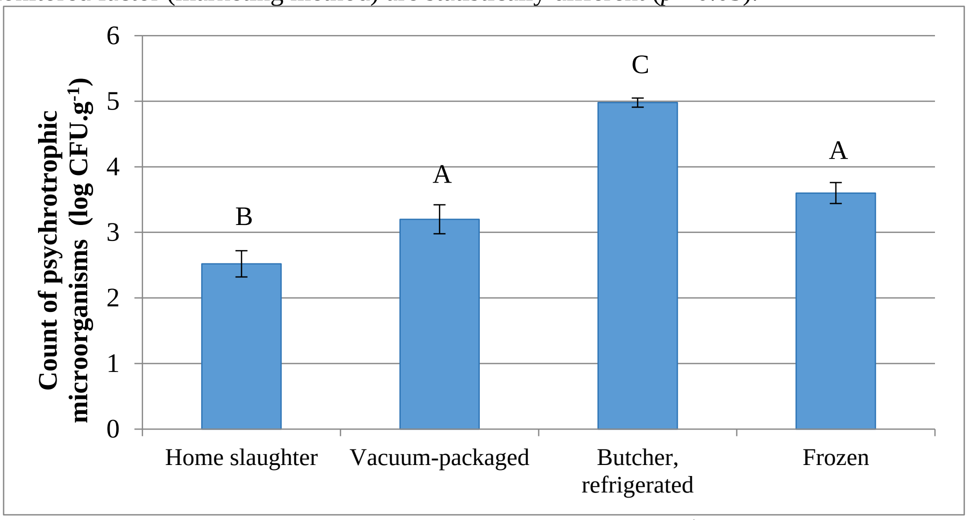

Figure 5 Comparison of counts of psychrotrophic microorganisms (log CFU.g^{-1}) in rabbit meat (n = 5). Averages marked with different letters in the monitored factor (marketing method) are statistically different (*p* <0.05).

the above mentioned experiment, increased to between 2.8 and 6.3 log CFU.g^{-1}. **Chabela et al. (1999)** found lower psychrotrophic counts after 14 days of storage (3.13 log CFU.g^{-1}).

The above results suggest that the rabbit meat shelf life can be increased for example by refrigeration, modified atmosphere or irradiation **(Berruga et al., 2005)**. Non-irradiated rabbit meat samples were found to be contaminated with relatively high initial counts of aerobic mesophilic bacteria, psychrophilic bacteria, enterobacteriaceae and molds and yeasts as their mean counts reached 6.02, 5.89, 4.79 and 4.89 log CFU.g^{-1}, respectively. Irradiation at 3 kGy reduced the counts of microorganisms from 94 to 99.7% **(Badr, 2004)**. Most

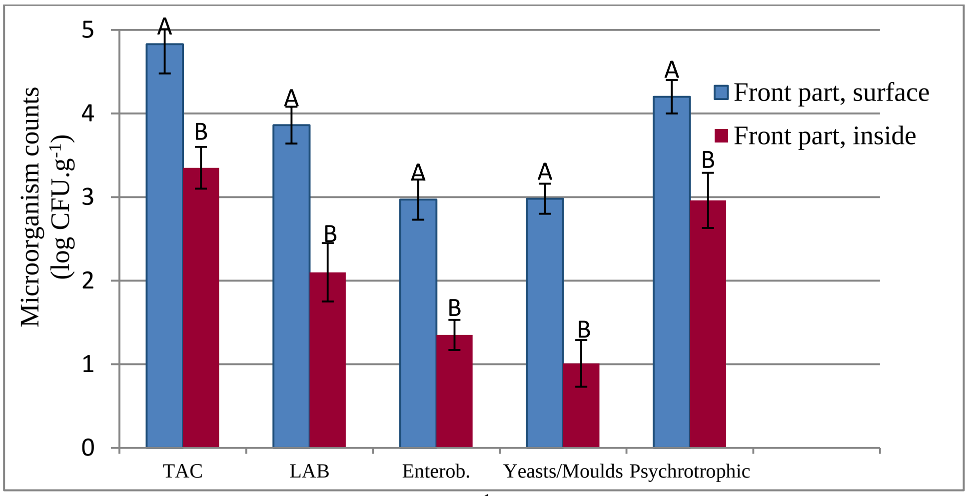

Figure 6 Comparison of microorganism counts (log CFU.g^{-1}) in rabbit meat (n = 20). Averages marked with different letters in the monitored factor (sampling site) are statistically different (*p* <0.05).

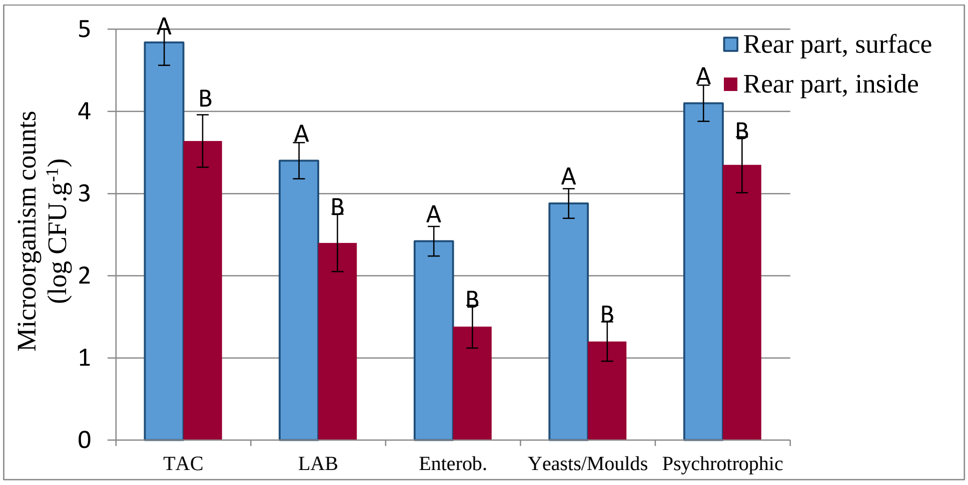

Figure 7 Comparison of microorganism counts (log CFU.g^{-1}) in rabbit meat (n = 20). Averages marked with different letters in the monitored factor (sampling site) are statistically different (p <0.05).

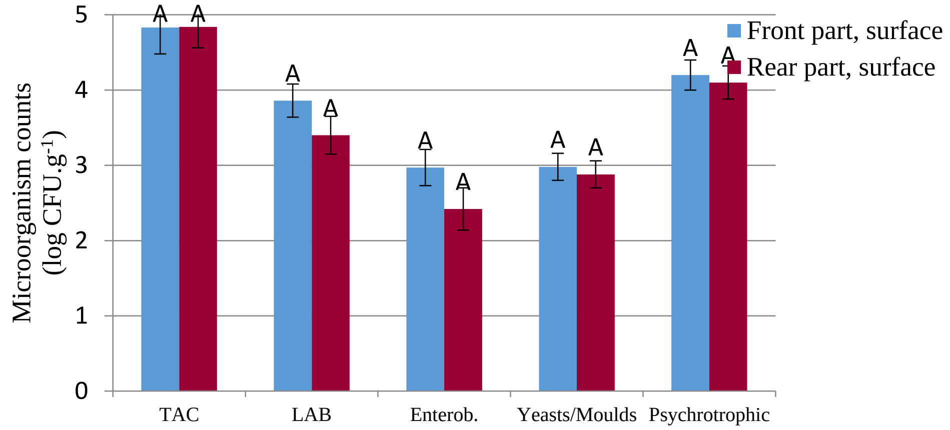

Figure 8 Comparison of microorganism counts (log CFU.g^{-1}) in rabbit meat (n = 20). Averages marked with different letters in the monitored factor (sampling site) are statistically different (p <0.05).

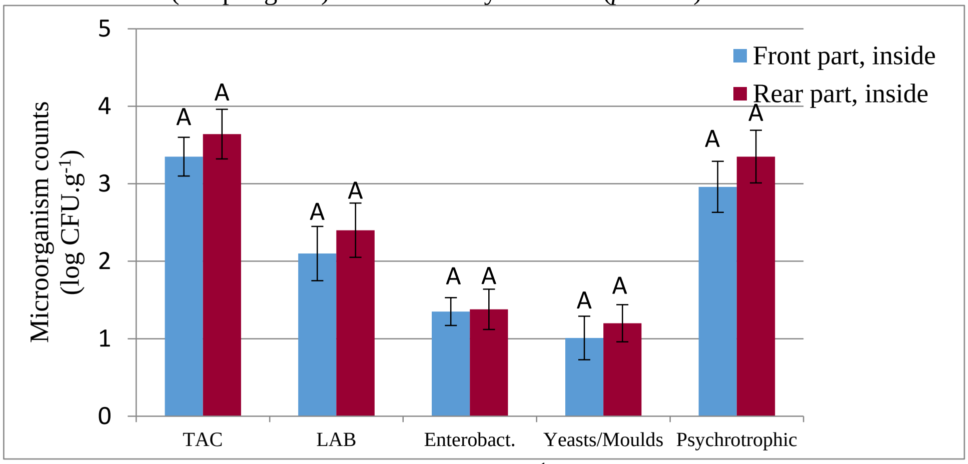

Figure 9 Comparison of microorganism counts (log CFU.g^{-1}) in rabbit meat (n = 20). Averages marked with differentletters in the monitored factor (sampling site) are statistically different (p <0.05).

important is, however, the compliance with the hygienic conditions during slaughter based on the HACCP principles **(Kohler et al., 2008)**.

Comparison of the count of microorganisms on the surface and within the muscle tissue of the front and rear parts of the rabbit carcass

In all our monitored microbiological indicators, was found differences (p <0.05) in their counts on the surface and within the muscle tissue, both in front of and rear parts of the rabbit carcass. In the front part of the carcass, the difference between the surface and the inside was 1.50 log CFU.g^{-1} on average (Figure 6).

The difference in the count of microorganisms between the surface and the inside of the rear part of the carcass averaged 1.20 log CFU.g^{-1} (Figure 7). This may be due to secondary contamination of rabbit meat from the air, from used tools, from skin and fur of animals, from containers, packaging materials, and crates **(Steinhauser et al., 2000)**. According to **Szkucik and Pyz-Łukasik (2009)**, bacterial contamination of the carcass surface in compliance with hygiene standards ranges from 3 to 4 log CFU.g^{-1}.

We have found values that are higher; in the front part of the carcass at 4.83 log CFU.g^{-1}. At the rear part of the rabbit carcass it was 4.84 log CFU.g^{-1} of mesophilic aerobic microorganisms. Lower aerobic total viable count of the haunch surface after slaughter found **Ludewig and Fehlhaber (2005)**, it was between log 3.77 and 3.80 CFU.g^{-1}.

Regarding the comparison of the microorganism counts in the rear and front parts of the rabbit carcass, both on the surface and inside the muscle tissue, was not recorded statistically significant difference in their counts at $p < 0.05$ (Figure 8 and Figure 9). **Abdel-Rahman et al. (2008)** also found no statistically significant difference in the count of microorganisms in the breast and thigh muscle parts, but they have studied chickens.

CONCLUSION

Based on these results, we can say that for maintaining the safety and quality of rabbit meat, it is most important to uphold rigorous hygiene not only during slaughter, but also during subsequent storage and handling. Although some ways of packaging and storage can to some extent prolong the shelf life and ensure satisfactory microbiological criteria, however, it always depends on the level of initial microbial contamination of meat. Therefore, in the context of the introduction of new EU legislation on food safety, rabbit slaughterhouses must apply control programs for safe and hygienic slaughtering conditions based on the principles of HACCP.

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