



eISSN 2239-7132

## Italian Journal of Food Safety

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Ital J Food Saf 2026 [Online ahead of print]

***Please cite this article as:***

Cantarini A, Limone F, Branciarri R, et al. **Microbiological quality and regulatory compliance of ground game meat produced in central Italy under Regulation (EC) No 2073/2005.** *Ital J Food Saf* doi:10.4081/ijfs.2026.14663

*Submitted: 18-11-2025*

*Accepted: 20-03-2026*

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## **Microbiological quality and regulatory compliance of ground game meat produced in central Italy under Regulation (EC) No 2073/2005**

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**Key words:** food hygiene, food safety, game meat, wild boar, roe deer, microbiological profile, minced meat.

**Contributions:** Andrea Cantarini: sampling and data analyses, manuscript original drafting. David Ranucci: study concept, manuscript original drafting. Raffaella Branciari, data analysis and interpretation, contribution to manuscript writing. Rossana Roila, Filomena Limone: sampling and data analyses; statistical analyses, contribution to manuscript writing and editing. Chiara Baldinelli, Federico Fiorucci: data interpretation, contribution to manuscript writing and editing.

**Conflict of interest:** the authors declare that they have no competing interests, and all authors confirm accuracy.

**Ethics approval and consent to participate:** this article does not contain any studies that would require an ethical statement.

**Availability of data and materials:** the data presented in this study are available on request from the corresponding author.

**Acknowledgments:** the authors would like to thank Giordano Angeli for his considerable support in the organisation of the research.

## Abstract

The establishment of structured supply chains for hunted game meat has fostered the development of a wide range of products intended for the food service sector. Among these, minced meat has gained particular prominence due to its practicality. Nonetheless, the grinding process can enhance microbial proliferation, posing potential challenges to product quality and safety. The hygienic profile of deep-frozen minced wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*) meat remains insufficiently characterized. A total of 70 minced meat samples (corresponding to 10 batches from wild boar and 4 from roe deer) were collected over a 1-year period from a dedicated processing facility. The samples were processed and subjected to aerobic colony counts (ACC),  $\beta$ -glucuronidase-positive *Escherichia coli* counts, and the detection of *Salmonella spp.* and *Listeria monocytogenes*. The mean ACC was  $4.64 \pm 0.75$  Log CFU/g, with no significant differences between wild boar ( $4.65$  Log CFU/g) and roe deer ( $4.61$  Log CFU/g) samples. Differences were observed for *E. coli* counts, with mean values of  $2.60 \pm 1.02$  Log CFU/g for wild boar and  $1.90 \pm 0.81$  Log CFU/g for roe deer. Neither *Salmonella spp.* nor *L. monocytogenes* was detected in any of the samples. When compared against the process hygiene criteria established by European legislation, 95.71% of the samples were classified as satisfactory and 4.29% as acceptable for ACC. However, with respect to *E. coli*, 35.71% of the samples exceeded 500 CFU/g, a level regarded as unsatisfactory for minced meat set in Regulation (EC) No. 2073/2005.

## Introduction

In recent years, consumer interest in the meat of wild ungulates has grown, even in territories traditionally less associated with hunting (Marescotti *et al.*, 2019). This trend may be explained both by the widespread perception that such meat is healthier (Tomasevic *et al.*, 2018) and by its organoleptic qualities (Kwiecińska *et al.*, 2017; Corradini *et al.*, 2022). Furthermore, some consumers are also motivated by ethical considerations, seeking a valid alternative to red meat derived from intensive livestock farming (Demartini *et al.*, 2018; Demartini *et al.*, 2021; Marescotti, *et al.*, 2020). Within this context, restaurateurs seek semi-processed products such as minced meat or minced meat preparations from game species to prepare a variety of dishes, and consumers increasingly demand culinary options that enhance the value of wild ungulate meat in innovative ways. However, due to its high nutrient content and water activity, minced meat is susceptible to significant microbial contamination and bacterial growth (Ercolini *et al.*, 2011).

It is important to emphasise that these products originate from carcasses of hunted animals that are processed within complex and frequently poorly standardised supply chains (Gaviglio *et al.*, 2017; Marescotti *et al.*, 2019; Peruzy *et al.*, 2022a), which may require specific regulatory adaptations to ensure consistency and safety (*e.g.*, microbial hygiene criteria for game meat carcass). Indeed, the regulation does not differentiate between ground meat obtained from farmed animals and that derived from hunted species, despite the substantial differences in the respective supply chains.

Vacuum packaging and freezing are common processes for ground meat, but there is limited information in the literature on the hygienic quality of the final product, particularly for game meat. To date, only two studies have been conducted on the hygiene level of ground meat from wild boar with high levels of aerobic colonies and *E. coli*, as well as foodborne pathogens (Kautto *et al.*, 2025; Sweden National Food Agency, 2025). However, these studies were performed in a different context from Italy, and there is no data available on roe deer ground meat. Accordingly, the objective of the present study was to evaluate the hygienic quality of vacuum-packaged, deep-frozen minced meat produced from wild boar and roe deer. *Salmonella spp.* and *Listeria monocytogenes* were also investigated as foodborne pathogens to assess some safety aspects of the products. In addition, the study compares the microbiological results with the process hygiene criteria set out in Regulation (EC) No. 2073/2005 (European Commission, 2005), which defines process hygiene criteria for minced meat regardless of the animal species.

## Materials and Methods

The study was carried out in the province of Perugia (central Italy), specifically within the Alto Chiascio area, between March 2024 and June 2025, on a local production chain. The animals were collected during selective and population control hunting, which was carried out by local hunters without the use of dogs. They were shot with monolithic bullets and transferred to a nearby collection centre within an hour, where they were eviscerated and inspected by a “trained person” in accordance with Regulation EC 853/2004. They were then stored at 4°C for a maximum of three days. The carcasses were transported under refrigerated conditions to the Game Handling Establishment, where the skin was removed on the same day. The carcasses were then checked and approved by the competent veterinary authority, after which they were stored for 24 hours at 7°C. In the event of abnormalities to organs and tissues detected by the “trained person”, the official veterinarian also carried out a post-mortem inspection of the viscera as required by European regulations. Subsequently, the carcasses were transported, while maintaining the cold chain, to the cutting plant authorized under Regulation (EC) No. 853/2004 (European Parliament and Council of the European Union, 2004), where it was promptly processed to obtain meat cuts to be sold as fresh or frozen meat. Shoulders and trimmings from the other cuts that were subjected to the grinding process. The processing temperature complies with the requirements set out in the current regulations for processing plants. The minced meat is usually vacuum-packed and deep-frozen at -40°C on the production line, ready for sale in 500 g or 1 kg packs. For this study, five samples of freshly ground game meat were collected from each daily production batch and deep-frozen at -40°C. The final samples for analytical purposes consisted of five sampling units of 50 g vacuum-sealed, deep-frozen packages (measuring 1 cm in thickness, 6 cm in width and 9 cm in length) for each batch. A total of 14 batches (10 from wild boar and 4 from roe deer) were analyzed, yielding 70 samples overall (50 from wild boar and 20 from roe deer). Samples were transported to the Microbiology Laboratory of the Department of Veterinary Medicine, University of Perugia, in an insulated container maintained at -18°C. Each sample was thawed overnight at 4±1°C before analysis, then 25 g of minced meat was diluted in buffered peptone water (Oxoid Ltd., Basingstoke, UK), followed by a homogenization with a Stomacher 400 Circulator (Seward Ltd., Norfolk, UK). Subsequent ten-fold serial dilutions were prepared in sterile physiological saline solution (Oxoid Ltd.). Aliquots were plated on selective media for aerobic colony counts (ACC), incubated aerobically at 30°C for 72 hours according to ISO 4833-1: 2013 (ISO, 2013) using Plate Count Agar (Biolife Italiana s.r.l., Milan, Italy). Enumeration of  $\beta$ -glucuronidase-positive *Escherichia coli* was performed on TBX medium (HiMedia™ laboratories Pvt Ltd.), incubated aerobically at 42°C for 24 hours following ISO 16649-1: 2018 (ISO, 2018). The same homogenized samples were incubated for one day at 37 °C to isolate *Salmonella* spp. according to ISO 6579-1: 2017 (ISO, 2017a). Subsequently, 0.1 mL of the pre-enriched solution was transferred into 9 mL of Rappaport-Vassiliadis broth (Biolife Italiana s.r.l.) and incubated again for 24±2 hours at 42°C to complete the enrichment phase. Following this step, isolation was performed by streaking the enriched broth onto a chromogenic agar selective for *Salmonella* (Biolife Italiana s.r.l.) using a sterile loop, and incubating the plates at 37°C for 24 hours. Qualitative detection of *L. monocytogenes* was carried out in accordance with ISO 11290-1 (ISO, 2017b). A 25 g sample was weighed and diluted in 225 mL of Half Fraser Broth (Biolife Italiana s.r.l.), then incubated at 30°C for 24±2 hours. Subsequently, 0.1 mL of the Half Fraser culture was transferred into 9 mL of Fraser Broth (Biolife Italiana s.r.l.) and incubated at 37°C for 48±2 hours. Finally, the culture was streaked onto ALOA (Oxoid Ltd.) plates with a sterile loop, and it was incubated at 37°C for 24±2 hours. Bacterial counts were expressed as colony-forming units per gram (CFU/g) and subsequently transformed into logarithmic (Log) values. The limit of detection (LOD) for *E. coli* was set at 1 Log CFU/g; therefore, samples without detectable colonies were assigned this value. The enumeration results were subsequently compared with the threshold values established by Regulation (EC) No. 2073/2005 (European Commission, 2005) regarding the process hygiene criteria for minced meat. For the per-batch confrontation, the results were compared according to the definition of satisfactory, acceptable and unsatisfactory defined in the regulation. Despite the regulations stipulating five sample units per

production batch, a per-sample comparison was also made to highlight the product's actual hygiene level. The hygiene criteria for the per-sample comparison are reported in Table 1.

Furthermore, the isolation and identification of foodborne pathogens were performed to determine the safety of the final product, despite it being intended for cooking as indicated on the producer's label.

A descriptive statistical analysis was performed on each batch and on the overall sample, with mean values and standard deviations reported. Differences between species were evaluated using an unpaired Student's t-test with a statistical significance threshold of  $p < 0.05$  (Microsoft Office Excel Statistic Data Tool Pack).

## Results

The results obtained for the enumeration of ACC and *E. coli* are presented in Table 2. Five samples were under LOD for *E. coli* (all belonging to roe deer samples). No significant differences were observed between wild boar and roe deer minced meat with respect to ACC; however, a significant difference was found for *E. coli* counts ( $p < 0.01$ ). None of the analysed samples tested positive for *Salmonella spp.* or *L. monocytogenes*.

No seasonal variation was detected in wild boar samples, both for ACC (average values 4.84 vs 4.46 Log CFU/g in autumn/winter and spring/summer season, respectively) and *E. coli* ACC (average values 2.48 vs. 2.66 Log CFU/g in autumn/winter and spring/summer season, respectively). Roe deer were hunted only during the spring/summer time.

The comparison between the data obtained for minced meat from individual species and the process hygiene criteria established by Regulation (EC) No. 2073/2005 (European Commission, 2005), on a per-batch basis reveals that, for ACC in wild boar, there were 8 satisfactory and 2 acceptable batches, and for *E. coli* enumeration, there were 3 satisfactory, 1 acceptable, and 6 unsatisfactory batches. All four batches of roe deer ground meat were satisfactory for ACC, while two of the *E. coli* batches were satisfactory and two were unsatisfactory.

The results obtained on a per-sample basis for this product are shown in Figures 1 and 2. As reported in Figure 1 for ACC, none of the samples was classified as unsatisfactory; 95.7% were deemed satisfactory, and 4.3% acceptable. All roe deer samples were classified as satisfactory, whereas 94% were satisfactory and 6% were considered acceptable in wild boar. For *E. coli* enumeration (Figure 2), 42.86% of the samples were classified as satisfactory, 21.43% as acceptable, and 35.71% as unsatisfactory. Specifically, 48% of the wild boar samples were unsatisfactory, compared with only 5% of the roe deer samples.

## Discussion

Overall, the ACC values were within the satisfactory and acceptable limits set out in EC Regulation 2073/2005 (European Commission, 2005), whether batch or single samples were considered. However, a substantial proportion of the samples exceeded the maximum limits established by the current regulation for *E. coli*, particularly those from wild boar. Only two studies have been reported in the literature that specifically evaluate the hygiene quality of vacuum-packed, deep-frozen minced wild boar meat. This study, conducted in Sweden, found that 25 out of 33 plants had unsatisfactory results for either ACC or *E. coli*, or both. The median values were 5.6 log CFU/g for ACC and 1.8 log CFU/g for *E. coli*, with significant variation between establishments (Kautto et al., 2025), and a high number of unsatisfactory samples from *E. coli* in ground wild boar meat (Swedish National Food Agency, 2025). These results were similar to those obtained in the present survey in Central Italy, which unfortunately relied on data from just one producer. With regard to game meat, one study assessed the hygienic status of frozen minced beaver meat. When compared with the findings of the present study, the results reported by Ziomek *et al.* (2021) exhibited higher ACC and lower *E. coli* levels. Although no other studies have specifically evaluated the hygienic status of minced meat from large game species, the results obtained in the present study can be compared with those reported by other authors for minced meat derived from livestock ungulates. Indeed, with regard to ACC, several

studies have reported higher counts than those observed in the present work, with values reaching up to  $6.8 \pm 1.0$  log CFU/g (Andritsos *et al.*, 2012; Bishop *et al.*, 2022), whereas for *E. coli*, the results obtained in this study appear, on average, higher than those reported in the literature, where the maximum values reached approximately  $1.4 \pm 0.7$  log CFU/g (Andritsos *et al.*, 2012; Bishop *et al.*, 2022). Some authors have investigated refrigerated whole muscle cuts from wild ungulates, finding considerably higher average values of both ACC (6.69 log CFU/g) and *E. coli* (3.02 log CFU/g) (Asakura *et al.*, 2017). Lower mean *E. coli* levels (2.03 log CFU/g) in both frozen and refrigerated meat cuts (forequarter and haunch) from wild boar and roe deer were reported by Membré *et al.* (2011), but slightly higher values (2.78 log CFU/g) were observed in the case of fresh stewing meat and roast beef cuts. It is important to note, however, that the last two aforementioned studies considered meat cuts from wild ungulates, for which no legal microbiological limits are established, unlike minced meat to which the process hygiene criteria set out in Regulation (EC) 2073/2005 (European Commission, 2005) can be applied.

In terms of food safety, it should be noted that no *Salmonella spp.* was isolated from the samples in the present work. In line with previous studies, the isolation of *Salmonella spp.* from wild ungulate carcasses appears to be sporadic (Bonardi *et al.*, 2021; Altissimi *et al.*, 2024; Floris *et al.*, 2024). However, other studies have detected *Salmonella spp.* in minced meat from domestic ungulates (Delhalle *et al.*, 2009; Zaiko *et al.*, 2021), with prevalence rates reaching 18% (Rašeta *et al.*, 2017). Furthermore, a report from the Swedish National Food Agency revealed the presence of foodborne pathogens such as *Salmonella spp.* and *Yersinia enterocolitica* in wild boar minced meat, in association with high *E. coli* counts (Swedish National Food Agency, 2025).

Regarding the detection of *Listeria monocytogenes*, none of the samples in the present study tested positive. This finding contrasts with previous outcomes on minced meat from livestock ungulates, with high prevalence rates up to 22% (Andritsos *et al.*, 2012; Andritsos *et al.*, 2013; Hadjilouka *et al.*, 2014).

The *E. coli* contamination emerged as the most critical issue observed in these samples, particularly in wild boar meat. It is well known that the method of carcass retrieval can be an influential factor; specifically, abdominal shots as well as carcass evisceration, which may result in gut rupture (Mirceta *et al.*, 2017). Indeed, particularly for ground meat, the initial hygienic conditions of the carcasses used as raw material are crucial. Several authors have evaluated the hygienic status of game carcasses by applying standards established for domestic ungulates and have reported higher levels of contamination compared to those found in carcasses from livestock (Avagnina *et al.*, 2012; Mirceta *et al.*, 2017; Orsoni *et al.*, 2020; Peruzzy *et al.*, 2022b). However, roe deer carcasses generally show lower contamination levels than wild boar ones (Obwegeser *et al.*, 2012; Branciarri *et al.*, 2020).

Moreover, the delay in carcass refrigeration is equally impactful. Regarding the production process, ground meat may also become contaminated during grinding. Nevertheless, the processing plant examined in this study is registered under Regulation (EC) 853/2004 (European Parliament and Council of the European Union, 2004), meaning it maintains an effective self-monitoring plan based on the implementation of the HACCP system. This ensures compliance with hygiene and safety standards through prerequisite programmes and critical control point monitoring. However, the facility operates on a small scale, processing only a limited number of carcasses per working day. For such a condition, in cases of unsatisfactory samples, it is quite difficult to reassess the origin of the raw materials, prescribed by legislation, together with proper sanitisation of the structures, in relation to the hunting availability. A proper formation of hunters, as well as of food business operators of the chain, could help improve hygienic production and reduce the need to be performed (at collection centres, game handling establishments, and transport) (Mirceta *et al.*, 2017; Abrantes *et al.*, 2023). The differences in hunters' formation and in the hunting practices for wild boar and roe deer could explain the results observed.

## Conclusions

The results of this study, even if preliminary and based on one single producer, suggest that ground meat from game meat may exceed the microbiological process hygiene criteria set out in legislation, particularly the *E. coli* threshold values for minced meat established in Regulation (EC) No. 2073/2005. This issue can be attributed to the complex supply chain of wild game and raises the question of whether the initial level of contamination can be considered a relevant factor in process hygiene, even when prerequisite programmes related to production hygiene are followed and shown to be effective. Further studies are needed to address hygiene and microbial contamination at every stage of the production process.

As compliance with Regulation (EC) 2073/2005 is mandatory for producers, and as the hygienic quality of raw materials can vary greatly due to factors such as the level of hunter training, products that commonly fail to meet process hygiene criteria may need to be excluded from production. Indeed, the producer can use the same cuts of minced game meat for ready-to-cook meat preparations, such as hamburgers, or for other meat products, that possess higher hygiene criteria thresholds set by European regulation.

In conclusion, for certain products, legal microbiological limits have previously been revised when the production of such foods could not reasonably be achieved under the existing criteria, accompanied by appropriate risk communication measures. In similar situations, the adoption of a risk-analysis approach would be necessary to ensure the safety of the final consumer.

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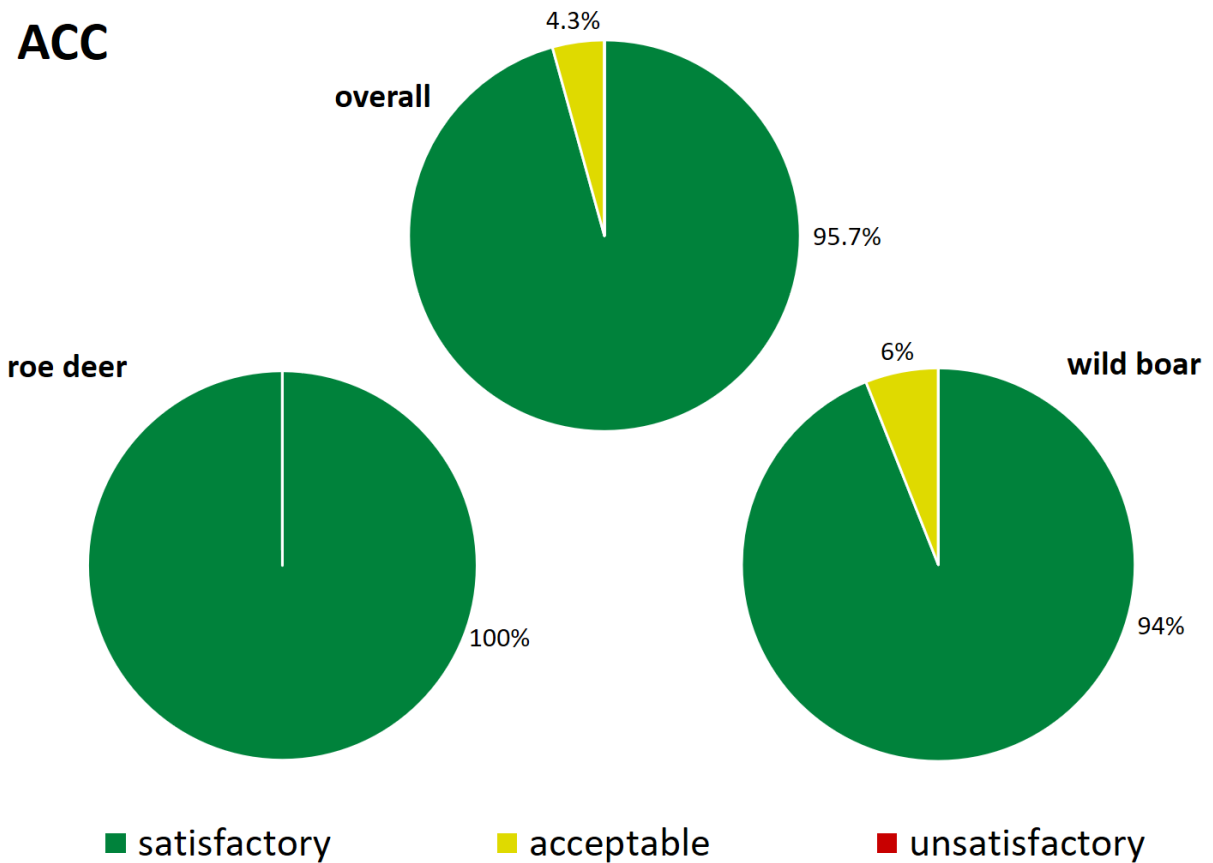
**Table 1. Hygiene criteria considered for minced meat in the per-sample comparison with Regulation (EC) N° 2073/2005.**

| CFU/g          | Satisfactory        | Acceptable                          | Unsatisfactory      |
|----------------|---------------------|-------------------------------------|---------------------|
| ACC            | $x < 5 \times 10^5$ | $5 \times 10^5 < x < 5 \times 10^6$ | $x > 5 \times 10^6$ |
| <i>E. coli</i> | $x < 50$            | $50 < x < 500$                      | $x > 500$           |

**Table 2. Mean values ( $\pm$  standard deviation) of vacuum-packaged, deep-frozen minced meat microbial counts (Log CFU/g).**

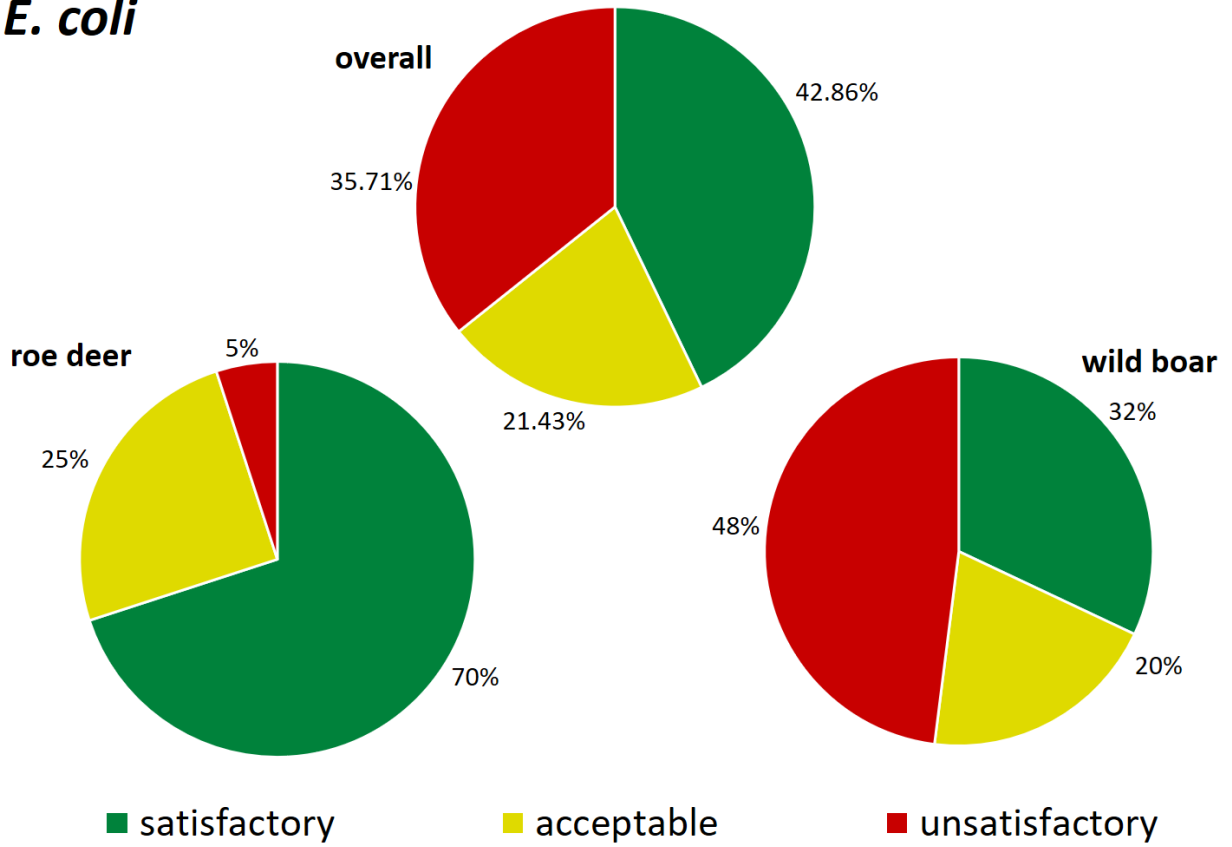
| Log CFU/g      | Wild boar         | Roe deer          | Total                |
|----------------|-------------------|-------------------|----------------------|
| ACC            | $4.65 \pm 0.74$   | $4.61 \pm 0.52$   | $4.64 \pm 0.75$      |
| <i>E. coli</i> | $2.60 \pm 1.02^b$ | $1.90 \pm 0.81^a$ | $2.40 \pm 0.91^{ab}$ |

n=70. Different letters (a, b) in the same row indicate statistically significant differences among mean values for the analyzed species (p<0.01).



**Figure 1. Percentage of compliance with Regulation (EC) No 2073/2005 limits set for aerobic colony count (ACC) of minced meat samples.**

***E. coli***



**Figure 2. Percentage of compliance with Regulation (EC) No 2073/2005 limits set for Escherichia coli of minced meat samples.**