Effect of time and temperature before chilling on the hygiene of carcasses in wild boar hunted in central Italy

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Abstract

The interest in certified game meat chains highlights the need for the evaluation and the management of factors affecting carcass hygiene along the peculiar steps of the production. The effects of time and temperature before chilling were specifically evaluated on aerobic colony count and *Enterobacteriaceae* count in hunted wild boar carcasses. Thirty wild boars were considered in two process steps where the hunted animal are still not chilled: after evisceration and just before chilling. Environmental temperature, carcass temperature and the elapse time between the two-step considered were registered. Furthermore, surface microbial loads were analyzed on the inner part of the carcasses. The mean time between the two sampling steps was 6 hours with an average environmental temperature of 20.49°C. A carcass temperature 9.6°C drop was observed during this period. In this lap of time aerobic colony count and *Enterobacteriaceae* count increased of 0.68 Log CFU/cm^2 and 1.01 Log CFU/cm^2 respectively, with a moderate correlation with the time but not with the temperature delta. The results reveal that the temperature conditions in central Italy hunting areas were not able to quickly reduce the carcass temperature and therefore the time between carcass evisceration and chilling should not exceed 6 hours.

Introduction

The hygiene of meat production is based on a structured chain that involves different roles and where procedures are properly defined even from a legislative point of view (EC Regulation 853/2004; Ranucci *et al.*, 2019; Primavilla *et al.*, 2021). "Farmed" animals are sent to slaughterhouses in good health state and are subjected to proper slaughter procedures under hygienic condition: stunned, quickly bled, properly skinned and eviscerated, eventually divided in half or parts and properly refrigerated without delay (EC Regulation 853/2004; Primavilla *et al.*, 2021). The production of wild game meat involves procedures that differ from those of "farmed" animals, and general guidelines are set by European and National legislations (EC Regulation 853/2004; Italian Government, 2021). The main differences between the two chains involve steps that are under hunters' control, such as the choice of the animal and the definition of its health status, the choice of the hunting day, the choice of the ammunition and hunting methods, the position of the shot, the bleeding and evisceration on field, the time and temperature before the arrival to a game handling establishment (Avagnina *et al.*, 2012; Gomes-Neves *et al.*, 2021; Mirceta *et al.*, 2017). The hunter is therefore responsible of the carcass hygiene in the harvest phase (Branciari *et al*., 2020; Orsoni *et al*., 2020; Ranucci *et al.*, 2021). A collection center for game meat nearby the hunting area, where carcass could be promptly eviscerated and refrigerated before sending to the game handling establishment, is not always available. For this reason, the carcasses are usually eviscerated on field and sent before refrigeration to the collection center for chilling or directly to the game handling establishment, for skinning and chilling. The time necessary for non skinned carcasses to be sent to the game handling establishment, and the relative environmental carcass temperature, could affects microbial loads (Paulsen and Winkelmayer, 2004, Vieira-Pinto *et al.*, 2014; Ranucci *et al.*, 2021). The EU legislation set that large wild game meat must be placed to the market only if the body is transferred to the game handling establishment "as soon as possible", and refrigeration must begin "after a reasonable period of time" after killing (EC Regulation 853/2004). In central Italy, the hunting of wild boar (*Sus scrofa* L.) is extremely popular and accessible for population control all over the year, even when environmental temperature is quite high (over 20°C) (Roila *et al.*, 2021). The aim of the study was to evaluate the effect of time and temperature, from evisceration to refrigeration, on the microbial growth in wild boars hunted in central Italy during temperate seasons (late summer and autumn). Moreover, the "reasonable period of time" for this specific contest is proposed.

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Key words: Game meat, Microbial load, Enterobacteriaceae count, Carcass hygiene.

Acknowledgments: The authors wish to express their gratitude to Filippo Molinaro at Centro di sosta Agonella ATC BO3 Sasso Marconi (BO), and Alessandro Monacelli at Serra Brunamonti S.r.l. Gubbio (PG) for their significant support in the organization and sampling of samples.

Contributions: The authors contributed equally.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Availability of data and materials: All the data have been presented within the manuscript.

Received for publication: 8 July 2021.
Revision received: 5 October 2021.
Accepted for publication: 20 October 2021.

Italian Journal of Food Safety 2021; 10:9959
doi:10.4081/ijfs.2021.9959

Materials and methods

The survey was conducted on thirty wild boars hunted in Bologna and Perugia Provinces, central Italy. The animals were all hunted for selection with a "still hunting" method (Ranucci *et al.*, 2021) properly shot to kill and eviscerated on field. After evisceration (average 1 hour and 18 minutes after the shot), the environmental temperature as well as the temperature of the carcass at the level of the *Gracilis* muscle were measured (FM12 Digital thermometer, Digitron, Frosinone, Italy) (T1), and a double swab samples (wet and dry swab, ISO 17604:2014) were collected from two inner surfaces on the right part of the carcass (superficial to the *Psoas major* muscle and superficial to the ribs at the level of the 9th thoracic vertebra). An area of 5x4 cm was delineated and sampled for each right surface considered, and the swabs were inserted in a propylene tube with 9 ml of sterile saline solution (Oxoid, Basingstoke, UK) and properly sent to the laboratory in refrig-

erated conditions. The carcasses were therefore sent to a collection center without refrigeration and, at the arrival, the same temperature measurements, and sampling (on the left inner parts) were performed (T2) and samples were sent to the laboratory. The difference between the body temperature values recorded at T2 and T1 was then obtained (Delta Temperature - ΔT). The time between the two sampling was also recorded. The samples were then properly diluted and analyzed for aerobic colony count (ACC) and *Enterobacteriaceae* count (ENT) according to ISO 4833-1:2013 and ISO 21528-2:2017, respectively. The results obtained were transformed into Log Colony Forming Unit (CFU)/cm^2. For samples that had counts below the detection limit (1 CFU/cm^2), a value of -0.05 Log CFU/cm^2 was assigned. The difference between the data registered at T2 and T1 was assessed (Delta counts - ΔC) for both ACC and ENT.

The ACC and ENT data performed on T1 and T2 were analyzed by an ANOVA model (one way ANOVA, Microsoft excel 2020). Tukey test were therefore performed, and significance was set at $p<0.05$. Pearson`s correlation was also performed between microbial loads and the other factors considered (time between T1 and T2, environmental temperature, ΔT) (Microsoft excel 2020). The samples were also divided according to two classes of time, between evisceration and refrigeration (below and above 6 hours), and data of the ΔC of these two classes were analyzed by the same ANOVA model previously reported.

Results

The average environmental temperature registered during the hunting days was 20.49°C (±2.72 standard deviation, s.d.) while the carcass temperature registered was 36.18°C (±2.77°C) and 27.02°C (±4.40°C) after evisceration and before chilling, respectively. Considering T1 and T2, the average drop of the temperature was 9.16°C (ΔT), while the mean time between the two steps was 6 hours and 15 minutes (range from 2.00 hours to 10 hours and 10 minutes, with 16 samples over 6 hours).

ACC and ENT counts, detected at the two sampling times considered, and the ΔC are reported in Figure 1. A significant increase of the loads was registered both for ACC ($p<0.05$) and ENT ($p<0.001$). A positive and significant correlation was registered between ACC values and time and environmental temperature, while correlation was detected only between ENT and time (Table 1). A positive correlation was also registered between time and ΔT (0.725, $p<0.001$) to highlight that even at relatively high environmental temperature, a reduction of body temperature occurs proportionally. Considering the average time of 6 hours detected between the two sampling, the ΔCs of both ACC and ENT of the two formed groups, below 6 hours (mean time of 3 hours and 37 minutes) and above 6 hours up to 10 hours (mean time of 8 hours and 24 minutes), are reported in Figure 2.

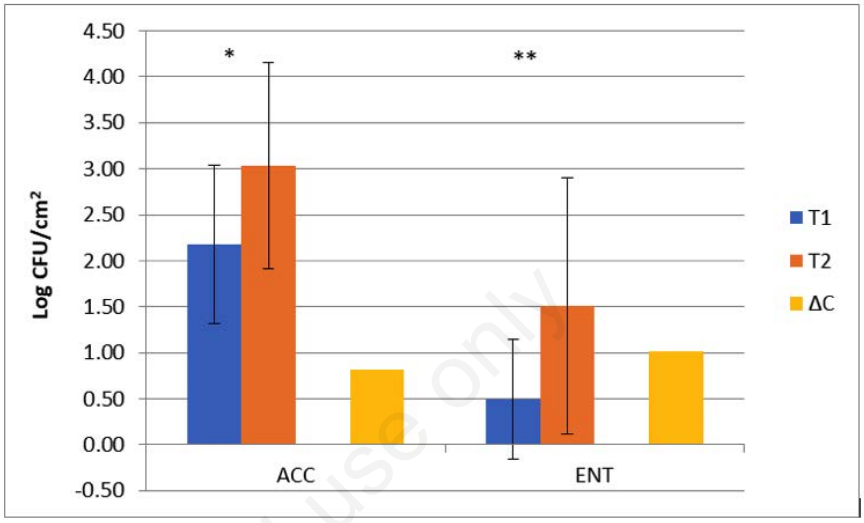

Figure 1. Aerobic Colony Count (ACC) and *Enterobacteriaceae* count (ENT) of the wild boar inner surface registered after evisceration (T1) and before chilling (T2), and average difference of the counts between samples collected at T2 and T1 (ΔC). Superscript on the bars define significant difference between the means (*p<0.05, **p<0.01).

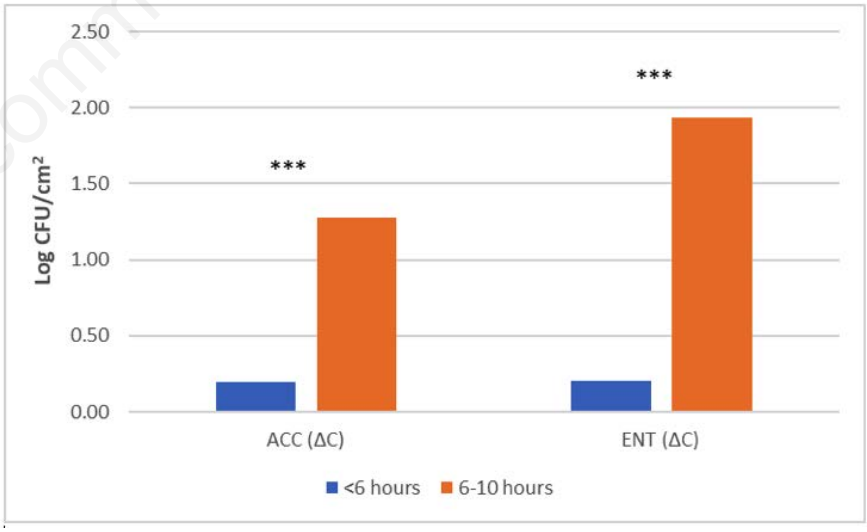

Figure 2. ΔCs for ACC and ENT before and after 6 hours from evisceration. Superscript on the bars defines significant difference between the data population (*p < 0.001).**

Table 1. Pearson's Correlation coefficients (and significance) between the microbial loads registered (ACC = Aerobic Colony Counts, ENT = *Enterobacteriaceae* count) and time or temperature parameters.

	Time	Environmental temperature	Body temperature drop
ACC	0.547 (p=0.0018)	0.485 (p=0.0007)	0.176 (p=0.352)
ENT	0.567 (p=0.0011)	0.280 (p=0.133)	0.081 (p=0.671)

The difference was significant between the two groups, with higher ΔC on carcass sampled above 6 hours after evisceration.

Discussion

The results highlight that the hunted wild boar in this specific geographic context (central Italy), are often sent to a collection center or to a game handling establishment under environmental temperature that are higher than those reported in literature for other regions. In the Alps, for instance, the temperatures reported during hunting are generally lower (Avagnina *et al.*, 2012) and their impact on the carcass hygiene, mainly on ACC, are reported when the hunting days are conducted above 15°C (Stella *et al.*, 2018). The prompt on-field evisceration when the hunting temperature is high could speed up the reduction of body temperature and therefore limit the bacterial loads of the carcasses (Winkelmayer *et al.*, 2008). The delay in the evisceration process could affect the carcass hygiene, due to the persistence of heat inside the body but also to gas formation potentially implying complications in the evisceration step (Avagnina *et al.*, 2012). Moreover, low level of hygiene of the carcass must be considered when the hunter is not properly formed on the best evisceration procedures to be adopted, especially on-field (Paulsen 2011; Avagnina *et al.*, 2012; Mirceta *et al.*, 2017). Performing wild boar evisceration after three hours after shooting is nonetheless considered to be critical (Winkelmayer *et al*., 2008). In the reported context of central Italy, even when evisceration is conducted on-field, a prompt refrigeration of the animal is recommended and could be achieved through the presence of available collection centers near the hunting areas (Ranucci *et al.*, 2021), as the degree of microbial load increasing is correlate to the time between evisceration and refrigeration. When environmental temperature is near or over 20°C, even if a proportion between the temperature drop and time between the two step was registered, the body temperature drop before chilling is inadequate, and the carcasses remain at a temperature that allow bacteria to growth (27°C). In fact, both ACC and ENT report an increase between T2 and T1 with ΔC near 1 Log CFU/cm^2. Other authors report that evisceration and skinning have to be performed within 6 hours from the shot (Decastelli *et al.*, 1995). Nonetheless, when the environmental temperature is particularly high, from early spring to late autumn in central Italy, a quick and hygienically accurate evisceration of the hunted wild boars on-field or in a collection center (within two hours from the shot, Ranucci *et al.*, 2021) combined with a suitable refrigeration (Borilova *et al.*, 2016) to be performed within 6 hours from the evisceration, is strongly encouraged.

Conclusions

These preliminary results confirm the need for a proper definition of procedures to be adopted by the hunters to guarantee the hygiene of wild boar meat and therefore their shelf life. Among this, relevant information could be provided in relation to steps that are not dealing exclusively with the hunting phase itself, but also with the carcass management. Proper strategies to reduce the time between evisceration and refrigeration, especially in warm climate environments, must be concerted between hunters and the relevant food business operator of the game meat chain. The proposed "reasonable period of time" for the refrigeration of the carcasses is not exceeding 6 hours from the on-field evisceration.

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