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Impact of the pre-slaughter period on the contamination of bovine leather and the operational sanitary procedure for skinning on the quality and microbiological safety of the carcass

Impacto do período pré-abate na contaminação do couro de bovinos e do procedimento sanitário operacional da esfola na qualidade e segurança microbiológica da carcaça

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Highlights

Reduction of *Enterobacteriaceae* by > 90% in animals subjected to 23 h of rest.
High recovery of STEC and *Salmonella* spp. from bovine leather.
Proper skinning technique minimizes enteropathogens in the carcass.
Proper skinning technique facilitates compliance with microbiological standards.

Abstract

Sanitary procedures are essential in cattle slaughter processes to minimize microbiological hazards from meat consumption. This study aimed to verify the influence of the pre-slaughter period and the correct execution of the operational sanitary procedure (OSP) for skinning in the breast region on the counts of indicator microorganisms and the occurrence of enteropathogens in the hides and carcasses of cattle. Forty-eight animals were evaluated and divided into 12 clusters, half of which were allowed 13 h of pre-slaughter rest, while the other half were allowed 23 h of pre-slaughter rest. The presence of microbiological

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indicators, including *Salmonella* spp., *Listeria* spp., and Shiga toxin-producing (STEC), enteropathogenic (EPEC), and enterohemorrhagic (EHEC) *Escherichia coli,* were evaluated in superficial samples of leather and carcass in which the OSP for skinning of the chest was performed either correctly or incorrectly. There was no significant effect ($p > 0.05$) of the pre-slaughter period on the counts of total coliforms, *E. coli,* enterobacteria, and mesophilic aerobics in the hide or carcass, although, in the carcasses, this difference was 93.4% for mesophilic aerobics and enterobacteria in the group of animals subjected to 23 h of rest compared to those subjected to 13 h of rest. Regarding the correct execution of the skinning OSP, there was also no significant effect ($p > 0.05$) on the indicator quantifications, but in relation to the presence of enteropathogens, it was possible to proportionally identify more EPEC and STEC in carcasses subjected to the wrong OSP, as it was only possible to identify *Salmonella* spp. and EHEC in carcasses subjected to the wrong OSP. The correct execution of the OSP for skinning incision in the chest region of the animal reduced the microbiological risk of the carcasses for the presence of enteropathogens and facilitated compliance with the microbiological standards for the carcass.
Key words: Diarrheagenic *Escherichia coli.* Microbiological quality. Operational sanitary procedure. *Salmonella* spp.

Resumo

Procedimentos sanitários são fundamentais no processamento de abate de bovinos para minimizar perigos microbiológicos ao consumo da carne. Esse trabalho teve por objetivo verificar a influência do período pré-abate e da execução correta do procedimento sanitário operacional (PSO) da esfola na região do peito nas contagens de micro-organismos indicadores e na ocorrência de enteropatógenos no couro e carcaça de bovinos. Foram avaliados 48 animais, divididos em 12 pools, dos quais metade foi mantida em 13 e os demais em 23 horas de jejum pré-abate. Foram quantificados indicadores microbiológicos e pesquisados *Salmonella* spp., *Escherichia coli* produtora de toxina shiga (STEC), enteropatogênica (EPEC) e enterohemorrágica (EHEC), adicionalmente *Listeria* spp., em amostragens superficiais de couro e carcaça nos quais foi executado o PSO da esfola do peito de forma correta e não conforme. Não foi verificado efeito significativo (p>0,05) do período pré-abate nas contagens de coliformes totais, E. coli, enterobactérias e aeróbios mesófilos no couro ou carcaça, apesar de nas carcaças essa diferença tenha sido de 93,4% para aeróbios mesófilos e enterobactérias no grupo de animais submetidos à 23h de repouso em relação à aqueles submetidos à 13h. Em relação à execução correta do PSO da esfola, também não foi verificado efeito significativo (p>0,05) das quantificações de indicadores, mas em relação à presença de enteropatógenos foi possível identificar, proporcionalmente, mais EPEC e STEC em carcaças submetidas ao PSO errado, assim como só foi possível identificar *Salmonella* spp. e EHEC em carcaças submetidas ao PSO errado. A execução do PSO da incisão do couro na região do peito do animal de forma correta reduziu, portanto, o risco microbiológico das carcaças para a presença de enteropatógenos e favoreceu o atendimento de padrões microbiológicos da carcaça.
Palavras-chave: *Escherichia coli* diarreiogênica. Procedimento sanitário operacional. Qualidade microbiológica. *Salmonella* spp.

Introduction

Ensuring the quality and microbiological safety of foods of animal origin is essential to minimize the risk to consumers' health. Furthermore, it facilitates biosecurity in the production chain and increases the economic potential of the animal protein industry by overcoming international quality barriers.

Slaughter and other stages of beef production are intensively controlled, as are all butchery animal species. Unlike other products of animal origin, such as milk, fresh meat is not subjected to any treatment that eliminates the microbiological and/or parasitic hazards that can cause foodborne illnesses (Arquias & Seixas, 2021).

Quality management tools are continuously developed and inspected in meat industries, allowing greater control and monitoring of the entire production process (Koutsoumanis & Sofos, 2004). Operational sanitary procedures (OSP), in addition to several other programs, aim to limit or control contamination potentially intrinsic to a particular step or process in the production flowchart (Costa, 2018).

Before cattle slaughter, the animals are kept at rest, fasted, and provided a water diet (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2017) in the establishment, above all, to minimize the occurrence of a dark, firm, and dry (DFD) meat in association with practices of animal welfare that directly influence meat quality (Adzitey & Nurul, 2011; Carrasco-García et al., 2020). During this period, which can range from 8 h to 23 h, little is known about whether time interferes with leather and carcass contamination. As the animals are continuously sprayed with water to promote animal welfare (Ahsan et al., 2014), it is expected that the longer the pre-slaughter waiting period, the lower the contamination of leather due to the removal of dirt by water sprinkling in addition to the regular sprinkling bath with hyperchlorinated water before the animal enters the slaughterhouse.

However, this water can flow to the region of the alba line in the ventral portion of the animal, where a skin incision is made prior to skinning the carcass. Therefore, it is essential that the cattle skinning OSP is carried out correctly; this involves directing the knife-edge to incise the hide from the inside to the outside of the carcass, preventing external contaminants on the animal's hide from entering into contact with the carcass.

The present study aimed to verify the influence of the pre-slaughter period on the contamination of bovine leather and the impact of the correct execution of the OSP for skinning of the breast region on the quality and microbiological safety of the bovine carcass, characterizing pathogens of interest for public health and the control of beef quality.

Material and Methods

A total of 48 animals were evaluated during the pre-slaughter period and during slaughter in a slaughterhouse under federal inspection in Araguaína, Tocantins, Brazil, on July 21, 2020. The animals originated from the same property and were divided into two batches of 24 animals each (lots A and B). The animals were kept in separate slaughter/waiting pens from the moment of unloading to the moment of slaughter and under the same conditions of rest, fasting, and water diet,

according to the Regulation of Industrial and Sanitary Inspection of Animal Origin (RIISPOA) (MAPA, 2017).

The killing barnyard was in accordance with TOMO I: Cattle of the Ministry of Agriculture, Livestock, and Supply (MAPA) (MAPA, 2018a). In short, it was arranged in parallel and divided by 2 m high fences and had waterproof, non-slip, washed, and disinfected floors before and after each batch was placed. Furthermore, it was equipped with drinking fountains sufficient for 20% of the animals to have simultaneous access and automatic supply, with the entire volume of water changed for each housed lot. It also had 30 cm high sanitary cords.

In these barnyards, lots A and B were kept in pre-slaughter rests for 13 and 23 h, respectively. Considering the local climatic conditions during this period, the animals were continuously subjected to water aspersion to promote animal welfare.

According to the recommendations of MAPA (MAPA, 2018a), all animals were subjected to the regular procedures of the establishment until the moment of this experiment: a spray bath in hyperchlorinated water (5 ppm) supplied at a pressure of 3 atm for 5 min before slaughter, stunning using the percussive penetrative method, hanging in noria through the left hind limb, bleeding for 3 min, skinning and disarticulation of the distal part of the limbs, occlusion of the rectum, sawing off of horns, and skinning of the ventral region.

Immediately before skinning the breast region of each animal, a superficial sampling of ~100 cm^2 of the leather in this region was performed with the aid of a sterile cellulose sponge (3M Microbiology, St. Paul, MN, USA) previously hydrated with 10 mL of buffered peptone water (H2Op) (Acumedia, Baltimore, USA).

Batches A and B, with 24 animals each, were divided into two groups of 12 animals each. In the first group, the OSP for leather skinning was performed correctly, with the knife-edge directed from the inside to the outside of the carcass. In the second group, the leather incision procedure was performed incorrectly, with the knife-edge running from the outside to the inside, which should be avoided during slaughtering operations. After performing this OSP correctly and incorrectly, individual sampling of the carcass was performed with the aid of a hydrated sponge in the same area where the leather incision was made. Thus, the experimental design was a completely randomized design, with two independent factors (pre-slaughter resting time, 13 h and 23 h; operation, correct and incorrect skinning OSP). The variables considered were the counts of microorganisms indicating quality and the incidence of enteropathogens.

The samples were refrigerated and sent to the Food Microbiology Laboratory (LabMA) of the Federal University of North of Tocantins, Araguaína University Campus, Brazil, where they were immediately analyzed.

Animal samples from batches A and B and their subgroups (correct and incorrect OSP) were analyzed in pools of four animals each, optimizing the microbiological analyses. Thus, from batch A, six pools of leather samples from 24 animals with 13 h of pre-slaughter rest were analyzed: three pools of four carcasses each, in which the correct OSP was performed, and another three pools of four carcasses in which the OSP was performed

incorrectly. The same grouping was carried out for lot B, which included animals allowed 23 h of pre-slaughter rest.

The sponges that made up each pool were combined in a single sterile plastic bag and homogenized in a Stomacher for 180 s (Alnajrani et al., 2018). Serial decimal dilutions were performed in saline solution (0.85%) and peptone (0.001%).

Counts for mesophilic aerobics, enterobacteria, total coliforms (30ºC) and *Escherichia coli* were performed in Petrifilm AC, EB, and EC (coliforms and *E. coli*), respectively, according to the manufacturer's guidelines (3M Microbiology, St. Paul, MN, USA). For analysis of the results, the counts were converted into $\log_{10}$ using BioEstat software (version 5.0; Stat Soft Inc., Tulsa, OK, USA) and Student's t-test with $\alpha = 5\%$.

The investigation for *Salmonella* spp. was performed according to the International Organization for Standardization - ISO 6579 [ISO] (2002) method, and that for *Listeria* spp. was performed according to the ISO 11290 (ISO, 2004) method, both of which were modified. After the isolation of characteristic strains on plates, the isolates were recovered in brain-heart broth (BHI) (Acumedia), subjected to DNA extraction (Ribeiro et al., 2016), and confirmed by genus-specific PCR according to the primers and amplification conditions presented in Table 1. The PCR reactions were performed with a final volume of 25 µL, and the conditions for the elaboration of these reactions were the same as those described by Ribeiro et al. (2019).

For the analysis of viable but nonculturable (VBN) isolates of *E. coli,* a 1 mL aliquot from each pool was inoculated into 10 mL of EC broth, incubated at 45 °C, and subsequently streaked onto eosin blue methylene agar (Merck, Darmstadt, Germany), according to the method described by Oliveira et al. (2021), to demonstrate the real presence and increase the analytical sensitivity and recovery of isolates for molecular characterization. From each pool, ~30 isolates suggestive of *E. coli* were recovered and pooled with those recovered from the Petrifilm EC plates. In these isolates, the virulence factors eaeA, *stx*1, and *stx*2 were investigated, according to the methods described by Ribeiro et al. (2019), as presented in Table 1, to confirm enteropathogenic (EPEC), Shiga toxin-producing (STEC), and enterohemorrhagic (EHEC) *E. coli.* The search for the *stx*1 and *stx*2 genes was performed using a multiplex assay, and that for the *eaeA* gene was performed using a uniplex assay, as shown in Table 1.

Table 1
Genes and amplification conditions of PCR reactions

Microorganism	*Gene*	*Primers (5' - 3')*	*Size (bp)*	*Amplification conditions*	*Reference*
Salmonella spp.	invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	94 ºC-1 min 35x (94 ºC-1 min, 64 ºC-30 s, 72 ºC-30 s) 72 ºC-7 min	Shanmugasamy et al. (2011)
Listeria spp.	iap	ATGAATATGAAAAAAGCAAC TTATACGCGACCGAAGCCAAC	1,450 1,600	95 ºC-5 min 40x (94 ºC-45 s, 52 ºC-45 s, 72 ºC-2 min) 72 ºC-10 min	Chen and Knabel (2007)
Escherichia coli	eaeA	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384	94 ºC-3 min 32x (92 ºC-30 s, 59 ºC-30 s, 72 ºC-1 min) 72 ºC-1 min	Paton and Paton (1998)
	stx1	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	180	94 ºC-3 min 32x (92 ºC-30 s, 61 ºC-30 s, 72 ºC- 1min) 72 ºC-1 min	
	stx2	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	255		

Results and Discussion

The counts of quality-indicating microorganisms in the leather of the 48 cattle evaluated after 13 h (24 animals) and 23 h (24 animals) of pre-slaughter rest are shown in Table 2. Similar results were reported by Cevallos-Almeida et al. (2021) for mesophilic aerobes and *E. coli.* It was possible to verify that the rest period had no significant effect on the microbiological contamination of leather. It was expected that the longer the animals stayed in the waiting corral, the extent of contamination increased due to the greater intensity of contact or decreased due to the effect of constant water sprinkling that removed dirt from the animal surface. However, a significant effect of the rest period was not observed, similar to the observations of Cevallos-Almeida et al. (2021).

Table 1
Genes and amplification conditions of PCR reactions

		Repose period**		
Group	Variations*	13 h	23h	p-value
Coliforms at 30 ºC (CFU/mL)	Max.	2 x 106	2 x 105	
	Min.	105	105	
	Mean (SD)	7.5 (±8.5) x 105a	1.5 (±0.57) x 105a	0.19
Escherichia coli (CFU/mL)	Max.	2 x 106	2 x 105	
	Min.	<105	<105	
	Mean (SD)	7.5 (±8.5) x 105a	1.2 (±0.5) x 105a	0.2
Mesophilic aerobes (CFU/mL)	Max.	6 x 107	1,1 x 108	
	Min.	3 x 106	1,3 x 107	
	Mean (SD)	3.2 (±1.9) x 107a	4.2 (±3.7) x 107a	0.23
Enterobacteria (CFU/mL)	Max.	1,3 x 106	1,5 x 105	
	Min.	4 x 104	2 x 104	
	Mean (SD)	3.5 (±5.1) x 105a	9.1 (±5.6) x 104a	0.13

* Max. = maximum; Min. = minimum; SD = standard deviation.
** Values on the same line followed by the same letter do not differ from each other at the 5% significance level.

As verified by the high counts, gram-negative microorganisms, such as E. coli, present themselves in high counts regardless of the rest period. In this context, performing the skinning step correctly contributes greatly to the reduction of carcass contamination by pathogenic strains of E. Coli, which is frequently reported in cattle in association with correct evisceration (Koutsoumanis & Sofos, 2004).

E. coli, when present in foods in general, is a microorganism that indicates hygienic-sanitary quality and indicates the origin of direct or indirect contamination by feces and, potentially, by other enteropathogens. Furthermore, many colonies of this species have virulence factors, indicating the microbiological risk from its consumption (Gomes et al., 2016). Table 3 shows that it was not possible to establish the counts of these microorganisms owing to analytical limitations. Considering its importance to the food chain, Normative Instruction No. 60 of 2019, from the Agência Brasileira de Vigilância Sanitária [ANVISA] (2019), states that fresh beef from cattle cannot have E. coli counts higher than 102 CFU/g.

The effect of performing the correct or wrong OSP, dependent and independent of the different rest periods in each group of animals, is shown in Table 3. For total coliforms and E. coli, it was not possible to verify the impact of the correct OSP performance because no results above the detection limit (103 CFU/mL) were observed in any of the evaluations.

For both mesophilic aerobes and enterobacteria, no significant difference was observed in relation to the correct execution of the skinning OSP, although for the two groups of microorganisms, counts ≈20% higher were observed in the groups of animals in which the OSP was performed incorrectly, compared to the groups in which the procedure was performed correctly.

The group of animals subjected to 23 h of pre-slaughter rest had lower counts in the two groups evaluated, as shown in Table 3. Especially in relation to the counts of mesophilic aerobics, it was possible to observe that in the pools of animals allowed 23 h of rest, the average reduction in counts compared to the groups subjected to 13 h rest was 93.4% for both OSP-W and OSP-C. Although this reduction in counts did not represent a significant difference, it is possible to infer, based on the percentage of reduction reported previously, that a longer period of rest for the animals can produce carcasses with lower microbiological counts, potentially related to the longer period of water spraying on the animals in pre-slaughter corrals and the consequent reduction of leather dirt. Several works available in the literature have also reported the effectiveness of performing OSPs for the microbiological quality of carcasses (Eisel et al., 1997; Milios et al., 2014; Fasanmi et al., 2018).

In agreement, the counts of enterobacteria in the carcasses of bovines submitted to 23 h of rest were lower than the detection limit of 103 CFU/mL, while for the group of animals submitted to 13 h of rest, the counts were detectable.

The Brazilian legislation that regulates the microbiological criteria for meat in the market (ANVISA, 2019) regulates the maximum count of mesophilic aerobics at 106

CFU/g in its sampling plan. Pools of carcasses with superior results were observed only in the group of animals submitted to 13 h of pre-slaughter rest and when the OSP of breast skinning was performed incorrectly.

Contamination with Salmonella spp. was only verified in isolates suggestive of hides and carcasses with incorrect execution of the skinning OSP, as shown in Table 4. The isolation of Salmonella spp. in meat cannot be positive according to the health legislation of the Brazilian Ministry of Health (ANVISA, 2019). This microorganism can be present in the carcass, rectum, skin, floor, hands of handlers, and water from cattle slaughterhouses (Shaibu et al., 2021). In addition, the study by Calle et al. (2021) found that the estimated probability of detection of Salmonella in carcasses was almost six times higher in the dry season, the period in which the present work was carried out, compared to the rainy season.

Although only one isolate (2.6%) among those suggestive of Salmonella spp. was confirmed in pool 23, the presence of Salmonella spp. is a qualitative analysis, and therefore, regardless of the isolated quantity, its presence already indicates a risk for consumers. This same risk, present in large quantities in bovine hides, was not observed when skinning OSP was performed correctly, demonstrating that the correct execution of skinning OSP can positively influence the microbiological safety of the carcass and enable compliance with the health legislation for meat processing/slaugther (ANVISA, 2019).

A recent study by Gutema et al. (2021) showed that of the 8.6% of bovine carcasses that were contaminated with *Salmonella* spp., only a few were contaminated with the same strain as that isolated from rectal swabs of those animals. The authors concluded that, for the most part, contamination of carcasses by this microorganism was more related to cross-contamination than to contamination with the fecal content of the animal itself. This directly implies the importance of the quality of execution of the procedures related to the handling of the carcass inside the slaughter plant, demonstrated by the present work.

Normative Instruction No. 60 of 2018 (MAPA, 2018b) of MAPA, unlike the legislation that regulates the microbiological quality of meat in the market (ANVISA, 2019), tolerates the presence of Salmonella spp. in two samplings (c) of a total of 50 (n) to be carried out per annual cycle for the size of the establishment where the samples were collected. Thus, the isolation of this microorganism under these experimental conditions did not compromise the compliance of the carcasses with the sampling plan of IN60/2018.

Table 3

Quantifications of quality-indicating microorganisms in the breast region of the carcass of 48 bovines submitted to the correct (OSP - C) and wrong (OSP - W) operational sanitary procedure (OSP) after 13 h and 23 h of pre-slaughter rest

Group	Variations*	Repose period**				OSP independent of rest period	
		13h***		23h***			
		OSP - C	OSP - W	OSP - C	OSP - W	OSP - C	OSP - W
Mesophilic aerobes (CFU/mL)	Max.	2×10^5	1.8×10^6	7.5×10^3	9.5×10^3	2×10^5	1.8×10^6
	Min.	7.8×10^3	1.3×10^4	2.2×10^3	4.4×10^3	2.2×10^3	4.4×10^3
	Mean (SD)	$7.8\ (10) \times 10^{4a}$	$10^5\ (8.4 \times 10^4)^a$	$5.1\ (2) \times 10^{3a}$	$6.6\ (2.6) \times 10^{3a}$	$4.1\ (7.7) \times 10^{4a}$	$5.5\ (7.5) \times 10^{4a}$
Enterobacteria (CFU/mL)	Max.	10^3	3×10^3	$<10^3$	$<10^3$	10^3	3×10^3
	Min.	10^3	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$
	Mean (SD)	$10^3\ (10^3)$	$1.3\ (1.7) \times 10^{3a}$	$<10^3$	$<10^3$	10^{3a}	$1.2\ (1) \times 10^{3a}$

* Max. = maximum; Min. = minimum; SD = standard deviation.
** Values on the same line followed by the same letter do not differ from each other at the 5% significance level.
*** OSP - C = Correct operational sanitary procedure; OSP - W = Wrong operational sanitary procedure.

Table4

Identification and characterization of the pathogenicity of isolates suggestive of Salmonella spp., Listeria spp., and Escherichia coli, isolated from hides and from animal carcasses subjected to the correct (OSP-C) and incorrect (OSP-W) operational sanitary procedure (OSP) after different pre-slaughter resting times in a slaughterhouse in Araguaína, Tocantins, Brazil

Variations*	Sample	Repose period	*Salmonella spp.*			*Listeria spp.*			*Escherichia coli*						
									Total isolates	EPEC	STEC			Total	EHEC
										eaeA	*stx1*	*stx2*	*stx1 e 2*		
			n	invA	%	n	iap	%	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
1 to 6	Leather	13 h	119	81	68.1	28	0	0	180	3 (1.6)	11 (6.1)	6	0	17 (9.4)	0
7 to 9	OSP - C	13 h	38	0	0	0	0	0	91	7 (7.7)	1 (1.1)	2 (2.2)	0	3 (3.3)	0
10 to 12	OSP - W	13 h	41	0	0	3	0	0	90	2 (2.2)	48 (53.3)	0	0	48 (53.3)	1 (1.1)
13 to 18	Leather	23 h	121	17	14	24	0	0	180	3 (1.6)	24 (13.3)	10 (5.5)	2 (1.1)	36 (20)	0
19 to 21	OSP - C	23 h	29	0	0	0	0	0	90	2 (2.2)	1 (1.1)	0	0	1 (1.1)	0
22 to 24	OSP - W	23 h	39	1	2.6	5	0	0	90	0	0	1 (1.1)	0	1 (1.1)	0

The same effect of OSP execution can be verified by molecular characterization of the isolates suggestive of *E. coli.* Although several pools did not present counts of these microorganisms, their recovery was possible after the enrichment step to verify VBN, enabling their characterization and clonal isolation, such as in pools 10 to 12, in which 53.3% of the isolates were found to have the stx1 gene. Despite this, it was possible to verify different strains of diarrheagenic *E. coli* contaminating the hide and carcass, especially in the carcass pools in which the wrong OSP was performed, as shown in Table 4. In carcass pool 10, in which the wrong OSP was performed, it was possible to characterize only the EHEC isolates (*eaeA* and *stx1* genes) (Silva & Silva, 2005). This characterization of diarrheagenic *E. coli* more frequently in carcasses subjected to the wrong OSP, as well as the isolation of *Salmonella* spp. in this condition, reaffirms that the correct OSP is essential for improving the microbiological safety of carcasses. In relation to the pre-slaughter rest period, it was possible to observe that in pools 1 to 6 (9.4%), the frequency of positivity for STEC was lower than that in pools 13 to 18 (20%), as shown in Table 4. However, under both 13 h and 23 h of pre-slaughter rest, all pools showed at least one positive isolate for STEC.

According to IN60/18 (MAPA, 2018b), microorganisms with the potential to produce Shiga toxin should be monitored in bovine and swine carcasses as they are indicators of the quality and microbiological safety of carcasses. STEC strains are commonly isolated from cattle, and their identification in meat determines their destination for heat-treated products (MAPA, 2018b). Irrespective of the compliance or non-compliance with OSP, it was possible to characterize STEC in carcasses after enrichment, although only one isolate was identified as positive for the *stx2* gene in pool 19. Thus, other procedures, in addition to the OSP for skinning of the breast region, must be adopted so that it is possible to reduce STEC and promote greater microbiological safety of the carcass.

A literature review by Antic et al. (2021) suggests that some bovine hide treatments, such as chemical washes and microbial immobilization treatment with shellac, and beef carcass interventions, such as hot water and/or steam pasteurization and lactic acid washes, show a consistent reduction in aerobic bacteria and microbial indicators of fecal origin, as well as a reduction in the prevalence of naturally present pathogenic *E. coli* and *Salmonella.* Given the importance of these microorganisms for the microbiological safety of meat, it is essential that the processing, with the realization of OSPs, be carried out correctly and that the need for secondary treatments is minimized to offer the consumer a safe product.

Conclusion

There was no significant effect of the pre-slaughter period on the microbiological contamination of the leather and carcass of cattle, despite a considerable reduction in the total microbiological count of the leather of animals subjected to the longest rest period. The correct execution of the OSP for skinning incision in the animal's chest region reduced the microbiological risk for the presence of enteropathogens in the carcasses and facilitated compliance with microbiological standards for the carcass. Despite this, other sanitary measures can be intensified during

beef processing to reduce the frequency of pathogens in carcasses, ensuring products have minimal risk to the consumer.

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References

Adzitey, F., & Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences-a mini review. *International Food Research Journal, 18*(1), 11-20.

Agência Nacional de Vigilância Sanitária (2019). Instrução Normativa nº60 de 23 de dezembro de 2019, Estabelece as listas de padrões microbiológicos para alimentos. *Diário Oficial [da] República Federativa do Brasil, 249*(1), 133.

Ahsan, M., Hasan, B., Algotsson, M., & Sarenbo, S. (2014). Handling and welfare of bovine livestock at local abattoirs in Bangladesh. *Journal of Applied Animal Welfare Science, 17*(4), 340-353. doi: 10.1080/1088 8705.2014.905782

Alnajrani, M., Hanlon, K., English, A., Fermin, K., Brashears, M. M., & Echeverry, A. (2018). Comparing the recovery of indicator microorganisms from beef trimmings using swabbing, rinsing, and grinding methodologies. *Meat and Muscle Biology, 2*(1), 154-161. doi: 10.22175/mmb2017.09.0047

Antic, D., Houf, K., Michalopoulou, E., & Blagojevic, B. (2021). Beef abattoir interventions in a risk-based meat safety assurance system. *Meat Science, 182*, 108622. doi: 10.1016/j.meatsci.2021.108622

Arquias, R. K. F., & Seixas, D. B. C. (2021). *Riscos do consumo de carne não inspecionada e as principais características sensoriais analisadas pelos consumidores-revisão de literatura.* Universitário FG. https://repositorio.animaeducacao.com.br/handle/ANIMA/13584

Calle, A., Carrascal, A. K., Patiño, C., Carpio, C., Echeverry, A., & Brashears, M. (2021). Seasonal effect on *Salmonella,* Shiga toxin-producing E. coli O157: H7 and non-O157 in the beef industry in Colombia, South America. *Heliyon, 7*(7), e07547. doi: 10.1016/j.heliyon.2021.e07547

Carrasco-García, A. A., Pardío-Sedas, V. T., León-Banda, G. G., Ahuja-Aguirre, C., Paredes-Ramos, P., Hernández-Cruz, B. C., & Murillo, V. V. (2020). Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. *Asian-Australasian Journal of Animal Sciences, 33*(10), 1656-1665. doi: 10.5713/ajas.19.0804

Cevallos-Almeida, M., Burgos-Mayorga, A., Gómez, C. A., Lema-Hurtado, J. L., Lema, L., Calvache, I., Jaramillo, C., Ruilova, I. C., Martínez, E. P., & Estupiñán, P. (2021). Association between animal welfare indicators and microbiological quality of beef carcasses, including *Salmonella* spp., from a slaughterhouse in Ecuador. *Veterinary World, 14*(4), 918-925. doi: 10.14202/vetworld.2021.918-925

Chen, Y., & Knabel, S. J. (2007). Multiplex PCR for simultaneous detection of bacteria of the genus *Listeria, Listeria monocytogenes,* and major serotypes and epidemic clones of *L. monocytogenes. Applied and Environmental Microbiology, 73*(19), 6299-6304. doi: 10.1128/AEM.00961-07

Costa, V. F. (2018). *Avaliação dos procedimentos sanitários operacionais (PSO) de bovinos no segundo semestre de 2017 em um frigorífico do município de Formiga-MG.* Trabalho de conclusão de curso de graduação em Medicina Veterinária, Centro Universitário de Formiga, Formiga, Minas Gerais, Brasil.

Eisel, W. G., Linton, R. H., & Muriana, P. M. (1997). A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. *Food Microbiology, 14*(3), 273-282. doi: 10.1006/fmic.1996.0094

Fasanmi, O. G., Makinde, G. E. O., Popoola, M. A., Fasina, O. F., Matere, J., & Ogundare, S. T. (2018). Potential risk factors associated with carcass contamination in slaughterhouse operations and hygiene in Oyo state, Nigeria. *International Journal of Livestock Production, 9*(8), 211-220. doi: 10.5897/IJLP2018.0491

Gomes, T. A. T., Elias, W. P., Scaletsky, I. C. A., Guth, B. E. C., Rodrigues, J. F., Piazza, R. M. F., Ferreira, L. C. S., & Martinez, M. B. (2016). Diarrheagenic *Escherichia coli. Brazilian Journal of Microbiology, 47*(Suppl. 1), 3-30. doi: 10.1016/j.bjm.2016.10.015

Gutema, F. D., Abdi, R. D., Agga, G. E., Firew, S., Rasschaert, G., Mattheus, W., Crombe, F., Duchateau, L., Gabriël, S., & De Zutter, L. (2021). Assessment of beef carcass contamination with *Salmonella* and *E. coli* O 157 in slaughterhouses in Bishoftu, Ethiopia. *International Journal of Food Contamination, 8*(1), 1-9. doi: 10.1186/s40550-021-00082-1

International Organization for Standardization 11290-1 (2004). *Microbiology of food and animal feeding stuffs - horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1: detection method.* ISO 1996.

International Organization for Standardization 6579 (2002). *Microbiology of food and animal feeding stuffs - horizontal method for detection of Salmonella* spp. (4nd ed.). ISO.

Koutsoumanis, K., & Sofos, J. N. (2004). Microbial contamination of carcasses and cuts. *Encyclopedia of Meat Sciences, 67,* 1624-1629.

Milios, K. T., Drosinos, E. H., & Zoiopoulos, P. E. (2014). Food safety management system validation and verification in meat industry: carcass sampling methods for microbiological hygiene criteria - a review. *Food Control, 43,* 74-81. doi: 10.1016/j.foodcont.2014.02.041

Ministério da Agricultura, Pecuária e Abastecimento (2017). Decreto nº 9.013 de 29 de março de 2017, Dispõe sobre o Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. *Diário Oficial [da] República Federativa do Brasil, 62*(1), 3.

Ministério da Agricultura, Pecuária e Abastecimento (2018b). Instrução Normativa nº60 de 20 de dezembro de 2018. Estabelece o controle microbiológico em carcaça de suínos e em carcaça e carne de bovinos em abatedouros frigorífico. *Diário Oficial [da] República Federativa do Brasil, 246*(1), 4.

Ministério da Agricultura, Pecuária e Abastecimento (2018a). *TOMO I: Bovinos.* MAPA. https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-animal/empresario/copy_of_TOMODEBOVINO.pdf/view

Oliveira, M. S., Santos, I. G. C. dos, Dias, B. P., Nascimento, C. A., Rodrigues, É. M., Ribeiro, J. C., Jr., Alfieri, A. A., & Alexandrino, B. (2021). Hygienic-health quality and microbiological hazard of clandestine Minas Frescal cheese commercialized in north Tocantins, Brazil. *Semina: Ciências Agrárias, 42*(2), 679-694. doi: 10.5433/1679-0359.2021v42n2p679

Paton, A. W., & Paton, J. C. (1998). Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for *stx 1, stx 2, eaeA, enterohemorrhagic E. coli hlyA, rfb O111, and rfb O157. Journal of Clinical Microbiology, 36*(2), 598-602. doi: 10.1128/JCM.36.2.598-602.1998

Ribeiro, J. C., Jr., Silva, F. F., Lima, J. B. A., Ossugui, E. H., Teinder, P. I., Jr., Campos, A. C. L. P., Navarro, A., Tamanini, R., Ribeiro, J., Alfieri, A. A., & Beloti, V. (2019). Short communication: molecular characterization and antimicrobial resistance of pathogenic Escherichia coli isolated from raw milk and Minas Frescal cheeses in Brazil. *Journal of Dairy Science, 102*(12), 10850-10854. doi: 10.3168/jds.2019-16732

Ribeiro, J. C., Jr., Tamanini, R., Soares, B. F., Oliveira, A. M., Silva, F. G., Silva, F. F., Augusto, N. A., & Beloti, V. (2016). Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. *Semina: Ciências Agrárias, 37*(5), 3069-3078. doi: 10. 5433/1679-0359.2016v37n5p3069

Shaibu, A. O., Okolocha, E. C., Maikai, B. V., & Olufemi, O. T. (2021). Isolation and antibiogram of *Salmonella* species from slaughtered cattle and the processing environment in Abuja abattoirs, Nigeria. *Food Control, 125,* 107972. doi: 10.1016/j.foodcont.2021.107972

Shanmugasamy, M., Velayutham, T., & Rajeswar, J. (2011). *Inv*A gene specific PCR for detection of *Salmonella* from broilers. *Veterinary World, 4*(12), 562-564. doi: 10.5455/vetworld.2011.562-564

Silva, J. A., & Silva, D. (2005). Escherichia coli Enteropatogênica (EPEC), ao contrário da *Echerichia coli* comensal, adere, sinaliza e lesa enterócitos. *Revista Patologia Tropical, 34*(3), 175-196. doi: 10.5216/rpt.v34i3.1925