Effect of different concentrations of chlorine, during pre-slaughter stage, on the count of hygiene indicator microorganisms in bullfrog (Lithobates catesbeianus) carcasses.

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In Brazil, the bullfrog (Lithobates catesbeianus) is classified as fish by the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin and must be slaughtered and processed under an official inspection regime and following the precepts of humane slaughter and hygienic-sanitary control. It is known that frogs are possible reservoirs of foodborne pathogens, such as Salmonella spp., and it is essential to understand how meat contamination occurs. Thus, it would be possible to standardize the stages of slaughter and processing of amphibians through legislation, which does not yet exist in the country. That said, the present work aimed to evaluate the effect of different concentrations of chlorine, during pre-slaughter stage, on the count of hygiene indicator microorganisms in bullfrog carcasses. 60 samples of bullfrog carcasses were collected at two stages during slaughter: post-bleeding (A) and post-toilet (B). The bullfrog underwent prior treatment, being immersed for 10 minutes in buckets containing water (Cl0: control group / absence of chlorine) and different concentrations of chlorine (Cl5: 5ppm; and Cl65: 65ppm). The protocol was approved by the Ethics Committee on the Use of Animals (CEUA): 3601717/2022. Samples for microbiological analysis were obtained by carcasses rinsing with saline solution, and the techniques used to count hygiene indicator microorganisms were: pour plate with Plate Count Agar (PCA) for aerobic mesophilic (AM), Petrifilm TM EC for Escherichia coli (EC) and Coliforms at 35oC (C35). The counts were compared by analysis of variance with Tukey\'s multiple comparison test for differences among the samples of each group at collection points A and B, respectively ($P<0.05$). To compare the counts obtained between stages A and B, the paired t-test was used ($P<0.05$). Finally, the comparisons of C35 and EC occurrence were made using Fisher\'s exact test ($P<0.05$). In stage A of slaughter, a lower AM count was observed in carcasses subjected to the Cl65 treatment (2.0 (±0. 60) log10 CFU/g) compared to the Cl0 treatment (2.6 (±0.80) log10 CFU/g) ($P<0.05$). Furthermore, a positive effect was observed in the use of chlorine in stage B of slaughter. At this stage, the AM count in the Cl5 treatment was 1.6 (±0.65) and in Cl65 it was 1.7 (±0.77) log10 CFU/g, both lower than the count obtained in the treatment Cl0 (2.5 (±0.88) log10 CFU/g) ($P<0.05$). Furthermore, the AM count, which was intermediate in stage A for the Cl5 treatment, suffered a significant reduction for stage B ($P<0.05$). This result indicates that Cl5 ended up having a late effect in reducing the AM count and may have acted as a barrier to cross-contamination during the slaughter process. For C35, there was no difference observed between the treatments at slaughter stages A or B, and between the stages for any given treatment. The likely explanation for this finding may have been the low C35 count, which ranged from 23.31 to <1 CFU/g. As an alternative to the quantitative assessment of C35 and EC, a qualitative evaluation was conducted, and again, no significant influence of chlorine usage was identified ($P>0.05$). Given these results, it is concluded that the use of 5 ppm of chlorine in the pre-slaughter stage is the dosage that

should be recommended for use, considering its effectiveness in reducing microbial contamination and cost-benefit in relation to higher concentrations of chlorine.

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