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SLAUGHTER AND PRE-SLAUGHTER PROTOCOLS FOR RODENTS AND BIRDS USED FOR ANIMAL FEED

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PROTOCOLOS DE ABATE E PRÉ-ABATE PARA ROEDORES E AVES UTILIZADOS NA ALIMENTAÇÃO ANIMAL

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ABSTRACT – Humane slaughter is defined as a set of guidelines that guarantee animal welfare from reception to slaughter. Irregular handling results in stress and suffering, directly affecting the quality of the meat. In Brazil, the current legislation for the slaughter of rodents, which are considered laboratory animals or guinea pigs, aims at euthanasia aimed at teaching and scientific research animals, generating remains in the carcass that cannot be reused in the feeding of carnivorous animals. Humane slaughter regulations are only aimed at butcher animals. This work evaluates the quality of meat from rats, mice and quails slaughtered

using specific protocols in order to reduce stress. For the experiment, 45 animals were slaughtered using specific slaughter protocols, from pre-slaughter management to slaughter. The pH of the animals analyzed varied between 5.5 and 5.8, complying with the quality parameters described in the literature. These results corroborate that good pre-slaughter and slaughter practices, in addition to reducing the animal's stress and suffering, result in a quality carcass.

Keywords: Animal nutrition; Animal welfare; Bioethics; Humane slaughter; Slaughtering techniques.

RESUMO – O abate humanitário é definido como um conjunto de diretrizes que garantem o bem-estar animal desde a recepção até o abate. O manejo feito de forma irregular resulta em estresse e sofrimento, interferindo diretamente na qualidade da carne. No Brasil, a legislação vigente para abate de roedores, que são considerados animais de laboratório ou cobaias, visam a eutanásia direcionado para animais de ensino e pesquisa científica, gerando resquícios na carcaça não podendo ser reutilizados na alimentação de animais carnívoros. As normativas de abate humanizado estão direcionadas somente para animais de açougue. Este trabalho avalia a qualidade da carne de ratos, camundongos e codornas abatidos utilizando protocolos específicos a fim de diminuir o estresse. Para o experimento, 45 animais foram abatidos utilizando protocolos específicos para abate, desde o manejo pré abate até o abate. O pH dos animais analisados variou entre 5,5 a 5,8 estando em conformidade com os parâmetros de qualidade descritos na literatura. Esses resultados corroboram que boas práticas de pré abate e abate, além de reduzir o estresse e sofrimento do animal resultam em uma carcaça de qualidade.

Palavras-chave: Abate humanitário. Bem estar animal. Bioética. Nutrição animal. Técnicas de abate.

INTRODUCTION

Humane slaughter is defined as a set of guidelines that guarantee animal welfare from reception to slaughter (BRASIL, 2000). Irregular handling results in stress, directly interfering with the quality of the meat (GALLO, 1994; GRANDIN, 1994; GREGORY, 1994). To mitigate these negative effects, it is essential to develop protocols focused on the well-being of these animals from creation to slaughter (HUMANE SLAUGHTER ASSOCIATION: HSA, 2021).

In Brazil, the current legislation regarding the euthanasia of rodents, particularly those designated as laboratory animals or guinea pigs, is primarily geared towards teaching and scientific research. Commonly employed techniques involve the use of inhalational anesthetics, which may result in particle residue in the fur, or injectable anesthetics. Unfortunately, these methods leave residues, rendering them impractical for animal consumption and incurring high costs (CONCEA, 2023).

Carbon dioxide is an accepted method of slaughter, provided it is administered at appropriate dosages by a qualified professional. While this method also leaves residues, the gas being naturally present in the atmosphere means it is not considered harmful residue for predators consuming prey euthanized with Carbon Dioxide (CONCEA, 2023). It's worth noting that there are currently no established protocols for large-scale slaughter with the intention of using the carcasses for animal feed.

To avoid causing suffering during slaughter, the stunning or stunning technique is used, aiming to reduce suffering at the time of death (GRANDIN, 1997; VELARDE et al., 1998; VELARDE et al., 2003). In Brazil, there are humane slaughter regulations, but they are aimed at butcher animals (BRASIL, 2000). For slaughter for human consumption, there is a European Union requirement that all animals go through a stunning process. Stunning is a procedure that makes the animal remain insensitive to pain, until there is complete loss of brain activity, and other subsequent procedures can be carried out without causing pain or distress (EEC, 1993; GIL and DURÃO, 1985). Electronarcosis is the most

used method in Brazil for slaughtering rabbits, birds, pigs and sheep (HENCKEL, 1998; PRÃNDL et al., 1994) and consists of the application of controlled discharges specific to the animal. However, there is no study on the use of this technique to stun prey used to feed carnivorous animals.

This study aimed to assess the meat quality of slaughtered prey and verify whether the animals experienced any level of stress before or during the slaughter process, using the electronarcosis stunning method. The evaluation hypothesis is that the proposed pre-slaughter management will promote adequate animal welfare and the use of electronarcosis as a means of stunning will be efficient, without resulting in eyelid and corneal reflexes, and as a consequence, the pH of the meat will be within the normal levels proposed by the literature.

MATERIAL AND METHODS

The study was developed at the facilities of the Pantanal Biotério, which aims to supply whole frozen prey intended for feeding captive carnivorous animals supported by the CONCEA 01/2024. The Biotério Pantanal produces four species of prey: Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), European Rabbit (*Oryctolagus cuniculus*) and quail (*Coturnix corner*). These are divided by weight category, ranging from newborns to reaching the adult size of the species. This diversity of weight allows the feeding of several species of predators of different age groups.

All animals were kept in temperature-controlled environments according to the needs of the species. Rodents belong to the BP® lineage. This lineage is made up of isogenic rodents that consist of a lineage of genetically uniform populations, with genetic similarity of 95% and reaching 99%. They were genetically selected to be precocious animals with a high birth rate, produced solely to serve as food for carnivorous animals.

The animals were fed twice a day, with food specific to each species. Water was available ad libitum through automated drinking fountains.

For slaughter, the animals were subjected to stunning and were subsequently slaughtered using Carbon Dioxide and then positioned and placed in an ultra freezer for rapid freezing.

Before slaughter, all animals were fasted, remaining in quarantine for 6 hours without access to food and water. The animals were kept in the same environment and breeding box, only changing the wood shavings bedding in order not to cause changes in the environment, minimizing stress. At the end of the fast, these animals were taken to the slaughter wing, approximately 5 meters from the breeding wing, where they were subjected to stunning followed by slaughter. From the moment of taking the box from the breeding wing to the stunning took about a minute.

To assess whether the animals were stressed or uncomfortable before the slaughter process, some observations were made, mainly regarding the animals' hygiene. Rodents in normal situations perform grooming, which are self-cleaning habits or cleaning habits of their partners, indicating that the animals have balanced stress levels. Rodents subjected to routine changes or stress tend not to perform the behavior of grooming. An area that is more difficult to clean is the tail. In this way, we can use it as a parameter of animal welfare when the rodent promotes self-cleaning of its tail (NEVES, 2013).

The animals underwent electroanesthesia as a stunning procedure and were subsequently transferred to a slaughter chamber where they received doses of carbon dioxide. They were positioned in a stainless steel structure connected to the electroanesthesia apparatus. Following this, a small amount of water was sprayed on the animals before stunning. Electroanesthesia was administered through a discharge of 60 volts and 90 milliamperes, lasting approximately 2 seconds, applied to the dorsal region. The use of carbon dioxide is recognized as an appropriate method of slaughter, provided that proper dosages are employed by an experienced professional (CONCEA, 2023).

To assess whether the stunning method was effective, two methods were used: eyelid reflection and corneal reflection. It was touched with the fingertip to the eyelid, if the animal closed, the stunning method was not effective. A fingertip was touched to the animal's cornea, if the animal closed, the stunning methods were not effective.

After slaughter, the animals were placed in a standard BP® position and taken to an ultrafreezer that reaches temperatures between -50 to -80°C. After 3 hours, the carcasses were removed and placed in conventional freezers or cold rooms with an average temperature of -30°C.

One day after the material went to the conventional freezer, 15 animals of each species of different weights were randomly chosen (Table 1) (quails, mice, and rat). Approximately 3g of muscle tissue was extracted from each mouse or rat from the left thigh, weighed on a precision scale (0.01g) and the same was done with quails, but samples were taken from the chest.

The extracted muscle tissue was then individually crushed and added to a 100ml beaker with 10ml of distilled water. Subsequently, it was transferred to a stoppered tube and subjected to vortexing for 1 minute to ensure homogenization.

RESULTS AND DISCUSSION

After the 6-hour fasting process, pre-slaughter stress was analyzed through unrestrained observation. It was observed that all evaluated animals remained calm, showing no signs of fear. Some engaged in grooming, while others played, and all had clean tails. This indicates that despite the 6-hour fasting period, the animals did not alter their behavior and did not exhibit any signs of stress due to the proposed management.

Regarding the evaluation of the method adopted for stunning (electronarcosis), for all animals used in this study, there was no occurrence that indicated failure in stunning, with no type of eyelid or corneal reflex observed.

Carcass weight and pH data are presented in Table 1. For rat, pH ranged from 5.51 to 5.8 with an average of 5.68 and standard deviation of 0.092; for mice, the pH variation was between 5.5 and 5.8 with an average of 5.69 and standard deviation 0.085 and for quails it was between 5.5 and 5.8 with an average of 5.65 and standard deviation 0.089 (Figure 1, Table 2).

Table 1. Mean and standard deviation of Rat, Mice and Quails subjected to stunning using Electronarcosis and subsequently slaughtered using Carbon Dioxide.

Animal	Weight	pH	Animal	Weight	pH	Animal	Weight	pH
Rat	4g	5.79	Mouse	3g	5.71	Quail	8g	5.79
Rat	6g	5.68	Mouse	5g	5.5	Quail	12g	5.74
Rat	8g	5.56	Mouse	7g	5.71	Quail	15g	5.73
Rat	10g	5.79	Mouse	12g	5.74	Quail	17g	5.59
Rat	12g	5.68	Mouse	15g	5.66	Quail	19g	5.61
Rat	19g	5.57	Mouse	20g	5.66	Quail	25g	5.61
Rat	36g	5.51	Mouse	26g	5.79	Quail	30g	5.78
Rat	49g	5.68	Mouse	27g	5.8	Quail	39g	5.58
Rat	126g	5.61	Mouse	27g	5.76	Quail	41g	5.79
Rat	140g	5.69	Mouse	27g	5.55	Quail	76g	5.65
Rat	201g	5.8	Mouse	28g	5.68	Quail	95g	5.65
Rat	256g	5.8	Mouse	30g	5.71	Quail	130g	5.8
Rat	303g	5.76	Mouse	31g	5.57	Quail	200g	5.59
Rat	350g	5.67	Mouse	33g	5.69	Quail	254g	5.55
Rat	389g	5.69	Mouse	36g	5.65	Quail	297g	5.72

Figure 1. Mean and standard deviation of Rat, Mice and Quails subjected to stunning using Electronarcosis and subsequently slaughtered using Carbon Dioxide.

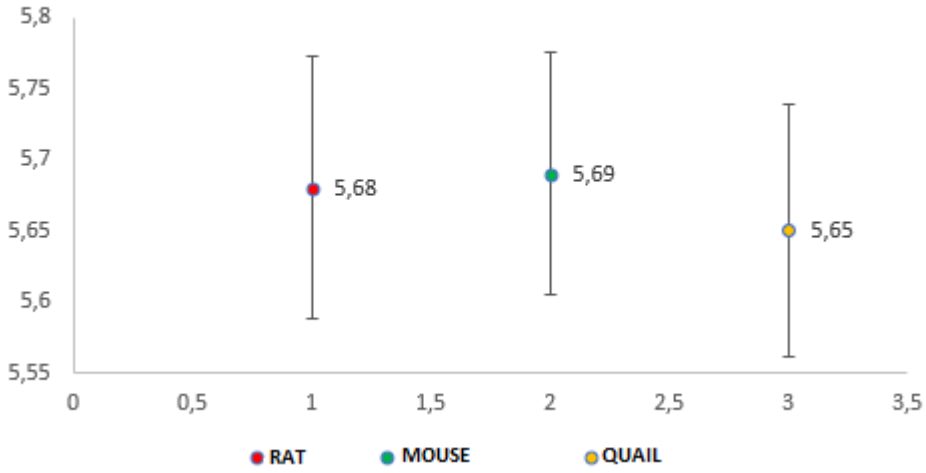

Table 2. Mean and standard deviation of Rat, Mice and Quails subjected to stunning using Electronarcosis and subsequently slaughtered using Carbon Dioxide. SD: standard deviation.

Animal	pH	SD
Rat	5.68	0.092
Mouse	5.69	0.086
Quail	5.65	0.089

Properly performed stunning depends on factors such as being carried out by a trained professional, equipment in good condition and properly calibrated. An animal stunned in the correct way collapses, falling to the ground, immediately after a shot. It then enters the tonic phase of the cerebral convulsion, with flexion and stiffening of the limbs lasting an average of 10 to 15 seconds after stunning. Then, the chronic phase begins, with paw movements similar to “pedaling” (GRANDIN, 1999). When pedaling does not occur, more attention should be paid to this animal, as it may return to consciousness, requiring further stunning (GREGORY, 2007). In addition to these signs, the corneal reflex is a great indication of a poorly stunned animal, as this reflex is the first to cease when the animal has been well stunned and the first to appear when the animal returns to consciousness (GREGORY, 2007). In the present study, no occurrence was observed that would indicate inadequate stunning, demonstrating the

effectiveness of using the electronarcosis method for rat, mice and quails, regardless of their size.

The pH of the animals analyzed varied between 5.5 and 5.8. A good rating for meat pH is between 5.5 to 5.8. Meats with a pH >6.0 are not considered suitable. In the case of beef, meat with a pH of 6.0 or more is considered DFD and cannot be exported. High pH levels in meat are caused by chronic stress before and during slaughter, leading to depletion of glycogen levels in the muscle. In this way, it prevents the conversion into lactic acid, which is responsible for the drop in pH. There is evidence that the main reason for DFD meat is inadequate handling and slaughter, causing physical exhaustion of the animal (ROÇA, 2001). With these results, the pre-slaughter methods and proposed slaughter methods become suitable as protocols to be used in the large-scale slaughter of rodents and quails used to feed carnivorous animals, maintaining the best quality of the meat and mainly guaranteeing animal well-being, reducing stress levels and reducing pain and suffering.

CONCLUSION

The combination of the proposed pre-slaughter management and the use of electronarcosis as a way of stunning rat, mice and quails were effective in promoting animal welfare and ensuring adequate meat quality of prey to be used as food for carnivorous animals. In addition to proving humane slaughter, without causing stress or pain to the animal, this work aims to propose slaughter techniques for animals used to feed carnivorous animals.

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