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COMPARATIVE STUDY ON THE VARIATION OF CORTISOL LEVEL IN BLOOD SERUM DEPENDING ON SWINE SLAUGHTERING METHOD

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Abstract

Stress is defined as a complex chain of events, consisting in a stimulus that causes a subsequent reaction in the brain and activates physiological reactions. It is important to adopt good practices during slaughter. In assessing the level of stress, the following variables should be taken into account: the means of transport, the way of slaughter (with or without stunning), accidental fall of animals, refusal of animals to enter the containment box, excessive movement of animals during containment. In the living organism, a series of biochemical and energetic transformations take place, which are in close interdependence, and are subjected to the mechanisms of regulation and metabolic control, which cease with the suppression of animal life, therefore, after slaughtering animals, a series of transformations appear in the muscle tissue. The study was conducted between 2019-2020, on two batches of conventionally slaughtered pigs (with stunning) in slaughterhouses and on a batch of traditionally slaughtered pigs (without stunning). In the slaughterhouses, the technological flow of pigs slaughtering was monitored and blood samples were collected in order to extract the serum and measure the cortisol level. Cortisol was measured in a specialized laboratory by the immunoenzymatic method by chemiluminescence detection. Determination of cortisol levels in blood samples taken from conventional pig slaughter revealed different values, exceeding the established reference values, compared with blood samples collected from households following traditional slaughter, the level of which is lower, sometimes falling within the reference values. The growth and handling of pigs before slaughter induces their stress, so special attention must be paid to the slaughter process in order to minimize stress levels and improve the quality of the meat.

Key words: *cortisol, pigs, stress, stunning.*

INTRODUCTION

Meat has played a crucial role in human evolution and is an important component of a healthy and balanced diet due to its nutritional richness (Savu et al., 2002; Williamson et al, 2005; McNeill & Van Elswyk, 2012; Pereira & Vicente, 2012; Petcu, 2013; Predescu et al, 2018). The nutritional composition of meat varies depending on the animal's breed, age, sex, diet, body weight, fattening status, rational feeding, animal health, animal movement, season, but also on the way of slaughter (with or without stunning) (Williams, 2007; Banu et al., 2009).

In pigs, in particular, there are a number of growth and fattening factors that affect the quality of the meat, such as: type of shelter, shelter size, microclimate in the shelter, animal density, feed, animal sex, age at slaughter, health, genetic factors, stressors and last but not least the weight at slaughter (Banu et al., 2009; Tăpăloagă, 2012).

In the living organism there are a series of biochemical and energetic transformations, which are in close interdependence, as they are subjected to the mechanisms of regulation and metabolic control, mechanisms that end with the suppression of animal life (Ionescu & Diaconescu, 2010).

After the slaughter of the animals, a series of transformations appear in the muscles tissue, as the blood pressure decreases, a peripheral vasoconstriction occurs, the thermoregulatory mechanisms no longer work, disturbances appear at the level of all homeostatic mechanisms and the susceptibility to microbial attacks increases (Ionescu & Diaconescu, 2010; Papuc et al., 2013; Petcu, 2015).

When the blood flow is interrupted, the oxygen supply is suppressed. Tissue respiration continues for a short time, until oxygen depletion. The absence of oxygen leads to the cessation of aerobic processes, and so the formation of lactic acid takes place through the anaerobic degradation of glucose. The accumulation of lactic acid in the muscles has the effect of decreasing the pH value, leading to its acidification (Ionescu & Diaconescu, 2010).

In order to obtain meat with physico-chemical characteristics corresponding to human consumption, animals are slaughtered, by different methods, depending on the species, religious precepts or geographical area. The process of sacrifice entails a series of consequences that have attracted the attention of the scientific world.

Stress is defined as a complex cascade of events, consisting of a stimulus (stressor), which causes a subsequent reaction in the brain (stress perception) and activates physiological reactions (stress response) (Dhabhar & McEwen, 1997; Ciliberti et al., 2017).

A stressor that lasts for a few minutes to hours is defined as acute stress, while a stressor that persists for several hours a day for weeks or months is defined as chronic stress (Dhabhar, 2002; Ciliberti et al., 2017).

Determination of cortisol is one of the most widely used methods of stress assessment in animals, because it provides information about the activity of the hypothalamic-pituitary-adrenal axis. The most frequently collected biological samples for cortisol dosing are: blood (serum, plasma), saliva, urine, feces, milk and hair (Casal et al., 2017).

MATERIALS AND METHODS

The study was conducted in 2019-2020 on three batches of pigs. The pig slaughtering technological flow was monitored in the slaughterhouses and blood samples were collected.

- Batch 1: 8 blood samples collected from a batch of 150 pigs, the Great White breed with an approximate body weight of 110-120 kg and the age of 8-9 months, slaughtered in a slaughterhouse, using stunning.
- Batch 2: 10 blood samples collected from a batch of 390 Metis breed pigs with an approximate body weight of 120-130 kg and the age of 7 months after slaughter in a slaughterhouse, using stunning.
- Batch 3: 12 blood samples collected from traditionally slaughtered pigs in the household of the population, the Metis breed, about one year old.

In the case of slaughter in the slaughterhouse, the pigs enter the adduction corridor and are electrically stunned, by positioning two electrodes at the level of the head. Immediately after stunning, hanging on the airline takes place and the next stage is bleeding.

In the case of traditional slaughtering, the pigs are slaughtered without stunning, by stabbing.

The aim of this study is to perform laboratory tests aimed at dosing cortisol from blood samples collected at the time of bleeding (approximately 9 ml of blood collected in a BD Vacutainer - Clot Activator Tube) (Figure 1). Blood samples were immediately transported to a specialized laboratory, and the cortisol level was dosed by the immunoenzymatic method by chemiluminescence detection.

In order to determine these parameters, specialized training and laboratory equipment, as well as specific materials and reagents are required.

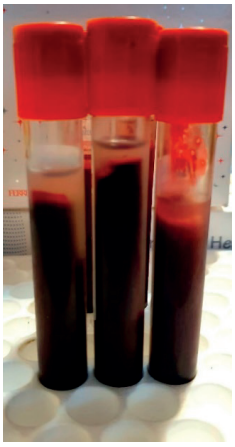

Figure 1. Blood samples

Animal welfare during transport and slaughtering is a matter of concern for consumers. It is necessary to pay attention to animals during transport, before slaughtering and during slaughtering (Petcu, 2015; Small & Hewitt, 2017).

People who understand the behaviour of animals will be able to board them properly into the means of transportation meant to carry them to the slaughterhouses, although stress is inevitable during the transport of animals from farm to slaughterhouse (Ferguson & Warner, 2009). Behavioural principles are recommended for the transport of animals, because this contributes to their welfare (Grandin, 2010; Grandin, 2019).

It is important to adopt good practices during slaughter, including systematic checks to determine when the animal begins to lose consciousness and when it loses it completely (Velarde & Dalmau, 2018).

In assessing the level of stress, the following variables should be taken into account: accidental fall of animals, refusal of animals to enter the containment box, excessive movement of animals during containment (Grandin, 2018).

Animals that become stressed before slaughter will have high levels of blood lactate concentration and will be more likely to have harder muscles. A calm animal that did not became restless and frightened will be more easily manipulated and will also be safer for the slaughterhouse staff (Grandin, 2010; Grandin, 2019).

The slaughtering without stunning is performed mainly for the purpose of religious sacrifice (Halal and Kosher), but also for the traditional sacrifice practiced in Romania for many years. If the animals are conscious during slaughtering, the risk of suffering increases. Immobilization of conscious animals for the purpose of cutting the neck causes stress. The incision made in the neck to cut the blood vessels, involves substantial damage to tissues in areas well represented by nociceptors (activation of the nociceptive system of protection induces suffering, pain in the animal). Death is not immediate and there is a period when the animal is still conscious and can feel anxiety, pain, suffering (Velarde & Dalmau, 2018).

Electrical stunning in pigs

Proper handling of animals during the slaughter process in well-designed units will minimize stress levels, improve efficiency and maintain good meat quality (www.grandin.com).

Handling in the last five to ten minutes before stunning the animals will have a significant effect on the blood lactate concentration. Studies have shown that high levels of lactate are associated with intense handling of animals that leads to stress. Also, improper electrical stunning of pigs and imposition of a second stunning leads to animal stress (Benjamin et al., 2001; Hambrecht et al., 2004; Hambrecht et al., 2005).

Slaughtered animals are stunned in order to enter a state of unconsciousness, insensitivity and immobility before bleeding. This state of unconsciousness should last long enough to ensure that the animal does not feel pain during bleeding (Wormuth et al., 1981; Schutt-Abraham, 1982; Gregory & Wotton, 1990; Hillebrand et al., 1996).

The effectiveness of the stunning process induces a state of instant unconsciousness and insensitivity to pain, which lasts until the death of the animal and has no negative effect on meat quality (Savenije et al., 2002; Joseph et al., 2013).

Animal welfare during slaughter was one of the major criteria that led to the formation of legislative requirements on stunning animals worldwide (Joseph et al., 2013).

Presently, the emphasis is on improving the animal's slaughtering process and new slaughtering procedures are followed, thus implementing various handling, stunning or monitoring techniques. The effectiveness and efficiency of stunning are of the utmost importance in facilitating the slaughter of animals, both for welfare and legislative reasons (Grandin, 2002; Atkinson et al., 2013; Grandin, 2019; Wagner et al., 2019).

Electrical stunning or electronarcosis is the passage through the brain of an electric current with voltage, amperage and frequency related to the species, which causes a disruption of normal brain activity, so that there is an immediate loss of consciousness and sensitivity. The efficiency of electronarcosis results from the interaction that is established between current, application time and the body's resistance. Practically, from the moment the two electrodes (positive and negative) are applied on the surface of the animal's body (Figure 2), the potential difference leads to the appearance of a current flow, with a certain

force, which will be counteracted by the resistance offered by skin (as a first obstacle) and the internal environment of the body (muscles, bones, blood vessels, etc.) (Guide on the protection of animals during slaughter, 2010; Petcu, 2015).

Figure 2. Electrical stunning in pigs

In all cases, the current level must be reached within one second from the start of the stunning and must be maintained for at least 1 to 3 seconds, according to the manufacturer's instructions. The electrodes must be placed so that they enclose the cranial box, allowing current to pass through it. The operator must ensure that there is a good electrical contact. In the case of pigs, the electrodes are located at the base of the ears, between the ears and the eyes. The alternating electric current, with low voltage is applied bilaterally, in the upper region of the skull, with the help of two electrodes of different shapes (Petcu, 2015).
Electrical stunning is based on the short-term action of electric current of a certain intensity and voltage on the central nervous system, causing paresis and loss of consciousness during the time in which the bleeding occurs (Petcu, 2015).
It is undeniable that an insufficient amperage or a current that after touching the animal's head takes it in another direction, without actually crossing the brain, will not induce the necessary state of unconsciousness, but pain caused by electric shock (Petcu, 2015).

RESULTS AND DISCUSSIONS

The period and method of slaughter are very complex and can represent different types of stress for the animal. How animals react to these stressors depends on their individual emotional reactivity (Deiss et al., 2009).
It has been shown that there is a direct correlation between meat quality and how animals are slaughtered (with or without stunning).

Results and discussions about the cortisol level in blood serum

Stress before slaughter has a negative impact on animal welfare and meat quality (D'Eath et al., 2010).
Determination of cortisol level is one of the most widely used methods for assessing stress in animals, as it provides information about the activity of the hypothalamic-pituitary-adrenal axis (Casal et al., 2017).
Deiss et al. has shown that the highest levels of cortisol (measured from blood samples) were observed in isolated animals. In general, young animals showed higher cortisol values (Linares et al., 2008; Deiss et al., 2009).
Determination of cortisol level in blood serum samples collected from conventionally slaughtered pigs revealed different values, exceeding the reference interval set by Jackson et al in 2002.

Study 1 - Determination of cortisol level from blood serum samples harvested from conventionally slaughtered pigs in June 2019

Following the analysis of the cortisol level from the 8 blood serum samples harvested from conventionally slaughtered pigs in June 2019, it was observed that 7 of the total samples had higher values compared to the reference interval (2.6-3.3 μg/dL), a single sample recording an optimal cortisol level, namely 2.80 μg/dL.
The results obtained from the dosing of cortisol level in the samples of group 1 are presented in Table 1.

Table 1. Results of cortisol level dosing in conventionally slaughtered pigs in batch 1

No.	Species	Breed	Age	Sex	Slaughtering date	Method	Cortisol level µg/dL	Reference interval
1.	swine	Large White	8 months	M	06.06.2019	Immunological	2.80 µg/dL	2.6-3.3 µg/dL
2.	swine	Large White	9 months	M	06.06.2019	Immunological	**3.73 µg/dL**	2.6-3.3 µg/dL
3.	swine	Large White	8 months	M	06.06.2019	Immunological	**4.39 µg/dL**	2.6-3.3 µg/dL
4.	swine	Large White	8 months	M	06.06.2019	Immunological	**4.89 µg/dL**	2.6-3.3 µg/dL
5.	swine	Large White	8 months	M	06.06.2019	Immunological	**5.51 µg/dL**	2.6-3.3 µg/dL
6.	swine	Large White	9 months	M	06.06.2019	Immunological	**6.21 µg/dL**	2.6-3.3 µg/dL
7.	swine	Large White	8 months	M	06.06.2019	Immunological	**7.10 µg/dL**	2.6-3.3 µg/dL
8.	swine	Large White	7 months	M	06.06.2019	Immunological	**7.99 µg/dL**	2.6-3.3 µg/dL

Study 2 - Determination of cortisol level from blood serum samples harvested from conventionally slaughtered pigs in November 2019

In November 2019, 10 blood samples collected from conventionally slaughtered pigs in a slaughterhouse were analysed in a specialized laboratory. All cortisol values obtained by analysis using immunological examination exceeded the reference interval. The lowest value recorded was 4.63 µg/dL and the highest value 16.0 µg/dL. The accepted reference interval is 2.6-3.3 µg/dL.

Sample number 7 registered a value 4 times higher compared to the reference interval, and sample number 8 registered a value 5 times higher. The results are presented in Table 2.

Table 2. Results of cortisol level dosing in conventionally slaughtered pigs in batch 2

No.	Species	Breed	Age	Sex	Slaughtering date	Method	Cortisol level µg/dL	Reference interval
1.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.23 µg/dL**	2.6-3.3 µg/dL
2.	swine	half-breed	7 months	M	18.11.2019	Immunological	**4.63 µg/dL**	2.6-3.3 µg/dL
3.	swine	half-breed	7 months	M	18.11.2019	Immunological	**6.83 µg/dL**	2.6-3.3 µg/dL
4.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.23 µg/dL**	2.6-3.3 µg/dL
5.	swine	half-breed	7 months	M	18.11.2019	Immunological	**8.15 µg/dL**	2.6-3.3 µg/dL
6.	swine	half-breed	7 months	M	18.11.2019	Immunological	**9.67 µg/dL**	2.6-3.3 µg/dL
7.	swine	half-breed	7 months	M	18.11.2019	Immunological	**12.7 µg/dL**	2.6-3.3 µg/dL
8.	swine	half-breed	7 months	M	18.11.2019	Immunological	**16.0 µg/dL**	2.6-3.3 µg/dL
9.	swine	half-breed	7 months	M	18.11.2019	Immunological	**9.34 µg/dL**	2.6-3.3 µg/dL
10.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.56 µg/dL**	2.6-3.3 µg/dL

Comparing the two groups analyzed, it can be seen that the highest values of cortisol levels were recorded in the group slaughtered in November, compared to the group slaughtered in June. This most likely correlates with the low temperatures to which the animals were exposed in the cold season, as temperature is, according to numerous studies, an important factor influencing the stress level of animals.

Guerrini and Bertchinger showed that the lowest plasma cortisol values were recorded during exposure of animals in a warm environment, and the highest values were recorded at the time of their exposure in a cool and moist environment. These results suggest that exposure of animals in a moist and low temperature environment causes an increase in cortisol concentration (Guerrini & Bertchinger, 1982).

Study 3 - Determination of cortisol level from blood serum samples harvested from traditionally slaughtered pigs in December 2020

The samples of study 3 were collected, following the traditional slaughter of pigs, in December 2020 in the period before Christmas, from the households of the population from Dâmboviţa county.

12 blood samples were studied, five of them obtaining an optimal cortisol level, and the other seven exceeding the values of the reference interval, but not as much as in the case of the results obtained from pigs slaughtered with stunning in the slaughterhouse, which most likely correlates with the growth method practiced, with the fact that the animals do not suffer from transport

stress and with the fact that the animals do not sit in crowded lots and do not feel the reactions of those slaughtered before them. The results obtained are presented in Table 3.

Table 3. Results of cortisol level dosing in traditionally slaughtered pigs in batch 3

No.	Species	Breed	Sex	Age	Weight	Slaughtering date	Growth system	Method	Cortisol level µg/dL	Reference interval
1.	swine	half-breed	M	12 months	160 kg	12.12.2020	Household	Immunological	3.00 µg/dL	2.6-3.3 µg/dL
2.	swine	half-breed	M	12 months	180 kg	13.12.2020	Household	Immunological	2.49 µg/dL	2.6-3.3 µg/dL
3.	swine	half-breed	M	12 months	200 kg	13.12.2020	Household	Immunological	2.37 µg/dL	2.6-3.3 µg/dL
„4.	swine	half-breed	M	12 months	160 kg	14.12.2020	Household	Immunological	2.12 µg/dL	2.6-3.3 µg/dL
5.	swine	half-breed	M	12 months	190 kg	14.12.2020	Household	Immunological	**6.12 µg/dL**	2.6-3.3 µg/dL
6.	swine	half-breed	M	12 months	160 kg	18.12.2020	Household	Immunological	**5.43 µg/dL**	2.6-3.3 µg/dL
7.	swine	half-breed	F	12 months	130 kg	18.12.2020	Household	Immunological	**6.24 µg/dL**	2.6-3.3 µg/dL
8.	swine	half-breed	M	18 months	350 kg	19.12.2020	Household	Immunological	**3.74 µg/dL**	2.6-3.3 µg/dL
9.	swine	half-breed	M	12 months	140 kg	19.12.2020	Household	Immunological	**5.14 µg/dL**	2.6-3.3 µg/dL
10.	swine	half-breed	M	12 months	220 kg	20.12.2020	Household	Immunological	**7.26 µg/dL**	2.6-3.3 µg/dL
11.	swine	half-breed	M	12 months	160 kg	21.12.2020	Household	Immunological	**4.20 µg/dL**	2.6-3.3 µg/dL
12.	swine	half-breed	M	12 months	200 kg	21.12.2020	Household	Immunological	2.41 µg/dL	2.6-3.3 µg/dL

Śmiecińska et al. in 2011 conducted a study on a batch of 24 pigs slaughtered immediately after transport and a batch of 20 pigs slaughtered after a 24 hour rest period. The cortisol level recorded an average value of 26.54 µg/dL in pigs slaughtered immediately after transport and an average value of 15.44 µg/dL in the group of pigs slaughtered after a rest period of 24 hours.

Batches 1 and 2 of the present study were slaughter after a rest period and had a mean cortisol value of 7.33 µg/dL, which is lower than the results of the above study, but which exceeds the reference interval. of 2.6-3.3 µg/dL.

Batch 3 represented by blood samples from pigs slaughtered in the traditional system, recorded an average cortisol level of 4.21 µg/dL, this being a value close to the maximum limit of the reference interval (2.6-3.3 µg/dL).

Increased cortisol levels are an indicator of the stress response of animals, resulting from the stimulation of the sympathetic and parasympathetic nervous system and the hypothalamic-pituitary-adrenal axis (Śmiecińska et al., 2011).

The above stimulates the adrenergic system to produce catecholamines and improves the secretion of steroid hormones, mainly cortisol, from the adrenal cortex (Zavy et al., 1992). Handling operations before slaughter induce an intense response to stress (Śmiecińska et al., 2011). At the same time, rest before slaughter physiologically balances the body and alleviates the stress induced by pre-slaughter manipulation (Gispert et al. 2000; Fischer, 2001; Śmiecińska et al., 2011).

The results obtained from the summary statistics (mean values and standard deviation) of blood samples collected are shown in Table 4.

Tabel 4. Summary statistics of cortisol level in blood serum samples (mean values and standard deviation) harvested from slaughtered pigs

Batch number	Cortisol (mean values and standard deviation)	Samples number
1	5.3275±1.73425	8
2	8.9340±3.267324	10
3	4.2100±1.785344	12

CONCLUSIONS

In the slaughterhouses from the study, all technological stages of animal slaughter are observed. No accidental fall of the animals on the supply corridor was observed, nor was their refusal to enter the containment box. The stunning method practiced is electric stunning.

Excessive handling of pigs before slaughter induces their stress, therefore special attention must be paid to the slaughter process in order to minimize stress levels and improve meat quality.

Respecting the rest period before slaughter physiologically balances the body and alleviates the stress induced by animals handling.

The highest values of cortisol levels were recorded in the batch slaughtered in November, compared to the batch slaughtered in June, which most likely correlates with the low temperatures to which the animals were exposed, as temperature is an important factor that influences the stress level of the animals.

Samples collected from traditionally slaughtered pigs obtained lower cortisol levels compared to blood samples collected from conventionally slaughtered pigs, which most likely correlated with the way the animals were grown, with the fact that they did not suffer from transport stress and the fact that the animals do not live in crowded batches.

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