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STUDY ON THE DIFFERENT LAMBS SLAUGHTERING METHODS AND THE ASSESSMENT OF THE DEGREE OF STRESS THROUGH THE DETERMINATION OF SERUM CORTISOL

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Abstract

All animals reared for meat will experience a level of stress prior to slaughter, which will manifest itself in negative effects on meat quality. The purpose of this research is to find out the level of stress of the animals, at the moment of slaughter, by measuring the cortisol in the serum, as an indirect method of evaluating the quality of the meat. The study was carried out between March and October 2021, on three batches of sheep. Batch 1 was slaughtered conventionally (with stunning), batch 2 was slaughtered traditionally (without stunning) and batch 3 was slaughtered halal (without stunning).). Blood samples were collected from the bleeding wound, and cortisol was dosed in a specialized laboratory by the immunoenzymatic method with chemiluminescence detection. Comparing the analyzed batches, it can be seen that higher average values of the cortisol level were recorded in the batch slaughtered in the halal system, followed by the batch slaughtered in the conventional system and then by the one slaughtered in the traditional system, which obtained the lowest values. The quality of the meat is directly influenced by the way the animals are slaughtered and by the stress during slaughtering.

Key words: *cortisol, halal, meet, sheep, stress.*

INTRODUCTION

Meat represents the skeletal muscle tissue of mammals, as well as the tissues with which it is in natural adhesions, such as: connective tissue, adipose tissue, bone tissue, nerves, lymph centers, blood vessels, etc. (Wood et al., 2003; Ionescu & Diaconescu, 2010; Petcu, 2013; Predescu et al., 2018).

In recent years, meat has become a controversial topic in public debates, as it involves several dimensions of sustainability. Here, we review trends in global meat consumption, including economic, social, environmental, health and animal welfare aspects (Poore & Nemecek, 2018).

Global meat consumption has increased enormously in recent decades. Notable increases in total consumption were observed in all regions of the world, but especially in Asia, Latin America and Africa. In terms of meat types, pork and poultry currently dominate global consumption and have seen the strongest increases in consumption. The increase in pork consumption is primarily driven by China and several other Southeast Asian countries. In contrast, poultry consumption has sharply increased in all parts of the world, because it is cheaper, often perceived as healthier, and also accepted by various religious precepts, than other types of meat (Mottet & Tempio, 2017). Although to a lesser extent, the global consumption of beef, sheep and goat meat, as well as other types of meat, has also increased over time (Parlasca & Qaim, 2022).

According to structure, pigmentation, chemical composition, meat is classified into (Figure 1):

- white meat, from poultry (chicken, turkey, ostrich etc.) and fish;
- red meat, coming from mammals (cattle, pigs, sheep, goats etc.);
- black meat, from game (Savu & Petcu, 2002; Ionescu & Diaconescu, 2010).

Meat is an important source of energy, and this is due to the high content of minerals (iron, potassium, zinc, phosphorus), but also of vitamins (vitamins from the B complex and vitamins A and D), thus representing a main component in human nutrition (Savu et al., 2002; Biesalski, 2005; Williamson et al., 2005; Petcu, 2015; Mihai et al., 2021).

Due to its chemical composition, meat is an excellent source of easily digestible proteins, being necessary for a balanced diet (Ionescu & Diaconescu, 2010; Mihai et al., 2021).

Parlasca and Qaim in 2022 mention that muscle tissue (meat) is a rich source of nutrients required for human nutrition, such as protein, vitamins and minerals, so meat consumption can help reduce nutritional deficiencies and promote human health (Headey et al., 2018; Zaharia et al., 2021).

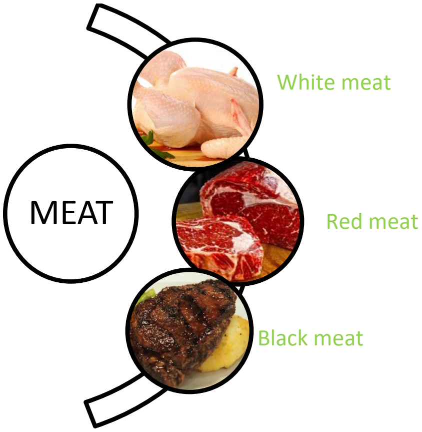

Figure 1. Types of meat available for human consumption

The transformation of animals for slaughter into meat takes place through a chain of events that include handling, loading, transport, unloading, preparation for slaughter and finally slaughter. During all these activities, due to incorrect handling, operational techniques and facilities, the animals are exposed to various stress conditions such as noise, vibration, restriction of movement, lack of food, adverse weather conditions, removal from the group, mixing with unfamiliar animals, overcrowding, increased human contact and inadequate pre-slaughter stunning, which can lead to unnecessary suffering, injury and production losses (Warris, 1995; Chambers et al., 2004; Birhanu, 2020).

Stress is a physiological disorder that is related to mental states associated with threatening or harmful and painful situations (Von Holleben et al., 2010). Stress before slaughter and during the slaughter stages can be differentiated into physical and psychological stress or both types of stress can be manifested (Lawrie, 1966; Mareko, 2005; Grandin, 2007; Adzitey, 2011; Chulayo et al., 2012; Birhanu, 2020; Barrasso, 2021).

Stress is the inevitable consequence of the process of transporting animals from the farm to the slaughterhouse (Ferguson & Warner, 2009; Mekibib et al., 2019).

Such a state of stress can lead to some changes in the concentration of enzymes (creatine-kinase, lactate-dehydrogenase, aspartate-amino-transferase, alanine-amino-transferase, alkaline phosphatase), hormones (catecholamines, cortisol) and other blood constituents , such as glucose and blood cell volume (Knowles & Warriss, 2007), resulting in muscle glycogen depletion, causing a lower rate of post-mortem lactic acid synthesis, high final pH, undesirable meat colour and higher water retention capacity (Chulayo et al., 2012). Furthermore, it causes carcass damage through bruising, hemorrhages, up to total carcass destruction and reduced live weight (Adzitey, 2011). Carcass damage results not only in an economic loss for the meat chain, but also a strong indicator of meat quality (Figure 2) (King et al., 2006; Adzitey, 2011).

The detection of stress in animals at the time of slaughter is necessary to manage the slaughter process in order to produce good quality meat (Ferguson & Warner, 2009; Pérez-Linares et al., 2015; Birhanu, 2020).

The slaughter process is complex, being characterized by a series of stressful stages, caused by many factors, therefore, the purpose of stunning is to render the animals unconscious during bleeding, without causing pain (Önenç & Kaya, 2004; Barrasso, 2021).

However, it is a common practice for Muslims and Jews to slaughter animals by a religious method, practiced without stunning (Önenç & Kaya, 2004; Barrasso, 2021).

Cortisol is a steroid hormone (glucocorticoid) released by the adrenal glands into the blood during stress period, and measuring its concentration in the blood is reliable and widely used as a good indicator of stress (Gupta et al., 2007; Novak et al., 2013).

The release of cortisol during stress also results in an increase in blood glucose through gluconeogenesis to aid in the rapid metabolism of carbohydrates, fats and proteins, nutrients

needed in stressful situations (Mostl & Palme, 2002; Birhanu, 2020).
The determination of cortisol is one of the most used methods for assessing stress in animals, and it can be measured from blood (serum or plasma), saliva, urine, feces, milk and hair (Casal et al., 2017; Mihai et al., 2021).
Hair cortisol analysis is a non-invasive procedure for detecting chronic stress, because cortisol is incorporated and stored inside the hair (Casal et al., 2017).

A recent study followed the cortisol level in sheep hair, harvested from different skin surfaces, following local treatments, such as: excessive brushing, the administration of hyperstimulating substances that increase blood circulation or a synthetic glucocorticoid. In this study, the dosage of cortisol concentration was carried out through immunological tests, an evaluation modality also applied in the present research (Salaberger, 2016).

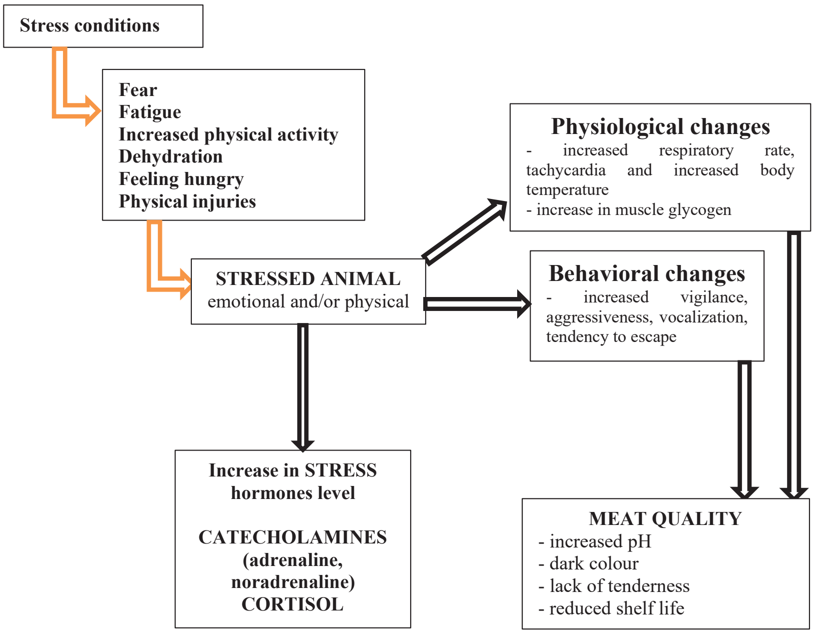

Figure 2. Effects of pre-slaughter stress on animals and meat quality

MATERIALS AND METHODS

The research was carried out between March and October 2021 on 3 batches of sheep, as follows: a batch of conventionally slaughtered sheep (with electric stunning), a batch of traditionally slaughtered sheep (without stunning) and a batch of halal slaughtered sheep (without stun). The following blood samples were collected:
- Batch 1: consisting of 15 blood samples collected from lambs reared on the farm, in March 2021, following conventional slaughter (with electric stunning), in an authorized sanitary-veterinary slaughterhouse.
- Batch 2: consisting of 15 blood samples collected from lambs in April 2021, during Easter, following the traditional slaughter in the households of the population.
- Batch 3: consisting of 15 blood samples collected from a batch of 60 farm-reared lambs of the metis breed, slaughtered in an authorized sanitary-veterinary and halal-certified slaughterhouse, in October 2021, without stunning, by the halal method, specific to Muslims.

In the slaughterhouses, the technological flow of slaughtering sheep was followed and blood samples were collected.
In the case of **conventional slaughter**, the sheep enter the adduction corridor and are electrically stunned, by positioning two electrodes at the head level. Immediately after the stunning, the hanging on the slaughtering

line takes place, and the next stage, namely the bleeding, will take place.

In the case of **halal sheep slaughter**, the animals are slaughtered without stunning, according to religious precepts. Each sheep is contained and the designated person at the slaughterhouse, cuts the blood vessels at the level of the animal's neck in a single movement, using a sharp knife. At the moment of bleeding, *Bismillah* is said.

In the case of **traditional slaughter**, practiced in Romania during the Easter holidays, the lambs are slaughtered without stunning, applying bleeding through the jugular.

The present study aims to carry out laboratory analyzes for the dosage of cortisol from blood samples collected at the time of bleeding (approximately 9 ml of blood collected in a BD Vacutainer - Clot Activator Tube). The blood samples were identified by labeling and transported immediately to a specialized laboratory, cortisol being dosed by the Immunoenzymatic method with chemiluminescence detection.

Cortisol was assayed from serum derived from blood samples (Figure 3) collected from the bleeding wound.

Figure 3. Blood samples collected from the bleeding wound and serum expression mode

For the determination of cortisol, specialized training and laboratory equipment, as well as specific materials and reagents, are required.

RESULTS AND DISCUSSIONS

The determination of the cortisol level in the blood samples collected after the slaughter of the lambs by different slaughtering methods, highlighted different values, and some of them exceed the reference range established by Jackson et al. in 2002, namely 1.40-3.10 µg/dL.

Results and discussions regarding the influence of the method of slaughter on the cortisol level in blood

Stress before slaughter has a negative impact on the hormonal system of animals and implicitly on meat quality (D'Eath et al., 2010; Mihai et al., 2021).

Study 1 - the influence of slaughter method on cortisol levels in blood collected from conventionally slaughtered lambs

Following the analysis of the cortisol level of the 15 blood samples collected from conventionally slaughtered lambs in March 2021, it was observed that 12 of the total samples had higher values compared to the reference range (1.40-3.10 µg/dL), and 3 samples recorded optimal values.

The highest value can be observed in sample number 8 (7.52 µg/dL), being 2.4 times higher than the maximum of the reference interval.

The results obtained after the cortisol dosage from the samples of batch 1 are presented in Table 1.

Table 1. Results obtained following cortisol dosing by immunological examination in lambs slaughtered in a conventional system from batch 1

No.	Slaughtering date	Sex	Age	Body weight	Cortisol level µg/dL
1.	25.03.2021	M	4 months	10 kg	3.02
2.	25.03.2021	M	4 months	11 kg	**5.25**
3.	25.03.2021	M	4 months	10 kg	**3.74**
4.	25.03.2021	M	4 months	12 kg	**4.93**
5.	25.03.2021	M	4 months	10 kg	2.08
6.	25.03.2021	M	4 months	10 kg	**6.41**
7.	25.03.2021	M	4 months	11 kg	**5.04**
8.	25.03.2021	M	4 months	10 kg	**7.52**
9.	25.03.2021	M	4 months	13 kg	**4.56**
10.	25.03.2021	M	4 months	10 kg	**4.86**
11.	25.03.2021	M	4 months	10 kg	**4.46**
12.	25.03.2021	M	4 months	10,5 kg	**4.89**
13.	25.03.2021	M	4 months	11 kg	2.46
14.	25.03.2021	M	4 months	10 kg	**6.06**
15.	25.03.2021	M	4 months	11.5 kg	**3.95**

Study 2 - the influence of slaughter method on cortisol levels in blood collected from traditionally slaughtered lambs

Following the analysis of the cortisol level of the 15 blood samples collected from traditionally slaughtered lambs in April 2021, it was observed that 2 of the total samples had higher values compared to the reference range (1.40-3.10 µg/dL), and 13 samples recorded optimal values. The average value obtained for all analyzed samples falls within the reference range, being 2.22 µg/dL.

The results obtained after the cortisol dosage from the samples of batch 2 are presented in Table 2.

Table 2. Results obtained following cortisol dosing in lambs slaughtered in the traditional system from batch 2

No.	Slaughtering date	Sex	Age	Body weight	Cortisol level µg/dL
1.	29.04.2021	M	4 months	10 kg	**5.69**
2.	29.04.2021	M	4 months	12 kg	**5.42**
3.	29.04.2021	M	4 months	12 kg	1.57
4.	29.04.2021	M	4 months	14 kg	1.91
5.	29.04.2021	M	4 months	10 kg	1.43
6.	29.04.2021	M	4 months	11 kg	1.48
7.	29.04.2021	M	4 months	12 kg	1.51
8.	29.04.2021	M	4 months	13 kg	1.63
9.	29.04.2021	M	4 months	14 kg	1.41
10.	29.04.2021	M	4 months	11 kg	1.40
11.	29.04.2021	M	4 months	15 kg	1.41
12.	29.04.2021	M	4 months	13 kg	1.42
13.	29.04.2021	M	4 months	15 kg	2.77
14.	29.04.2021	M	4 months	13 kg	2.52
15.	29.04.2021	M	4 months	14 kg	1.77

Study 3 - influence of slaughter method on cortisol levels in blood collected from halal slaughtered lambs

In October 2021, in a specialized laboratory, 15 blood samples collected from lambs slaughtered in the halal system in an authorized sanitary-veterinary slaughterhouse were analyzed by immunological examination. It can be seen that 13 of the total samples recorded values exceeding the reference interval (1.40-3.10 µg/dL), only two samples recording an optimal cortisol level. Sample number 10 recorded the highest value, namely 8.76 µg/dL.

The results obtained after the cortisol dosage from the samples of batch 3 are presented in Table 3.

Table 3. Results obtained after cortisol dosing in lambs slaughtered in the halal system from batch 3

No.	Slaughtering date	Sex	Age	Body weight	Cortisol level µg/dL
1.	20.10.2021	M	4 months	10 kg	**3.89**
2.	20.10.2021	M	4 months	11 kg	**7.25**
3.	20.10.2021	M	4 months	11 kg	2.41
4.	20.10.2021	M	4 months	10 kg	**5.43**
5.	20.10.2021	M	4 months	10 kg	3.08
6.	20.10.2021	M	4 months	10 kg	**6.31**
7.	20.10.2021	M	4 months	10,5 kg	**5.04**
8.	20.10.2021	M	4 months	10 kg	**3.52**
9.	20.10.2021	M	4 months	10 kg	**3.56**
10.	20.10.2021	M	4 months	11,5 kg	**8.76**
11.	20.10.2021	M	4 months	10 kg	**4.18**
12.	20.10.2021	M	4 months	10 kg	**4.25**
13.	20.10.2021	M	4 months	12 kg	**3.25**
14.	20.10.2021	M	4 months	10 kg	**4.55**
15.	20.10.2021	M	4 months	10 kg	**5.05**

Results and discussions regarding the statistical analysis of the data

The results obtained from the summary statistics (mean values, standard error of the mean (SEM), standard deviation, maximum and minimum values) of serum cortisol samples collected from slaughtered animals are presented in Figure 4.

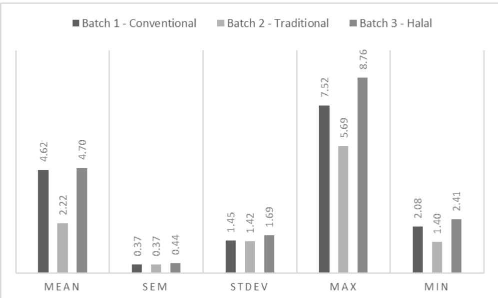

Figure 4. Summary statistics of serum cortisol level in lambs (µg/dL)

Comparing the analyzed batches, it can be seen that higher values of the cortisol level were recorded in the batch slaughtered in the halal system, followed by the conventional slaughtered batch and then by the traditional slaughtered batch.

Batch 1 was slaughtered in March, batch 2 in April and batch 3 in October, months with relatively similar temperatures, ambient temperature being a very important factor in inducing animal stress.

The highest value was recorded in a sample from group 3 (8.76 µg/dL), and the lowest value was recorded in group 2 (1.40 µg/dL).

The results for the three batches, were analyzed statistically by applying ANOVA one-way analysis of variance test, using the GraphPad Prism Statistical Software and the results obtained showed significant differences between the examined batches ($P<0.05$) (Figure 5).

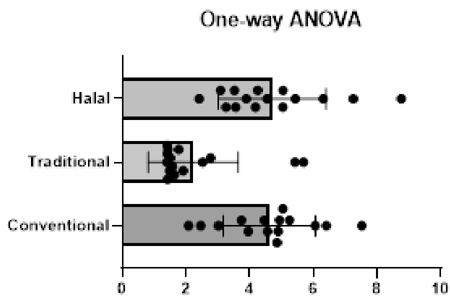

Figure 5. Summary statistics of ANOVA test

In order to determine the significance of the differences between the experimental groups, the t-test (student test) was applied.

The results obtained from the summary statistics of t-test (Student test) for all pairwise comparisons are presented in Table 4.

Table 4. Summary statistics of serum cortisol level in ovine - student test

Batches analyzed	P - value	Significantly different (P<0.05)	Mean ± SEM
Conventional - Traditional	0.0009	Yes	4.62 ± 0.3743, n=15 2.22 ± 0.3654, n=15
Conventional - Halal	0.8533	No	4.62 ± 0.3743, n=15 4.70 ± 0.4373, n=15
Traditional - Halal	0.0004	Yes	2.22 ± 0.3654, n=15 4.70 ± 0.4373, n=15

Batch 1 slaughtered conventionally and batch 2 slaughtered traditionally obtained statistically significant differences ($P<0.05$) (Figure 6).

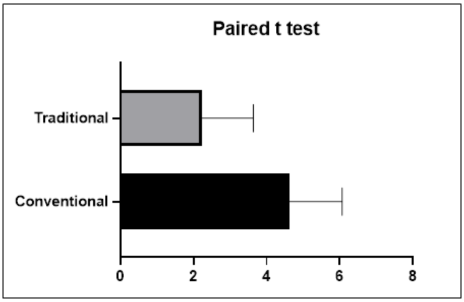

Figure 6. Mean value between batch 1 and batch 2

Batch 1 slaughtered conventionally and batch 3 slaughtered halal obtained statistically insignificant differences ($P>0.05$) (Figure 7).

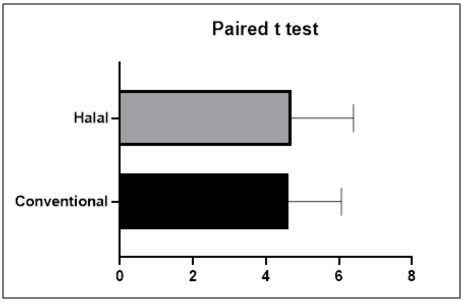

Figure 7. Mean value between batch 1 and batch 3

Batch 2 slaughtered traditionally and batch 3 slaughtered halal obtained statistically significant differences ($P<0.05$) (Figure 8).

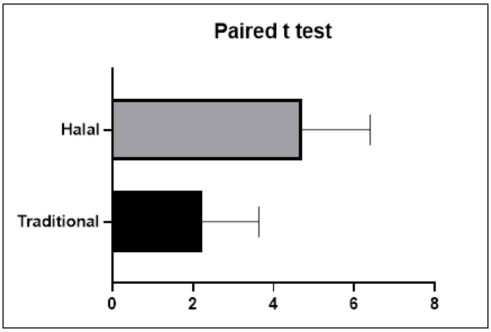

Figure 8. Mean value between batch 2 and batch 3

CONCLUSIONS

In the slaughterhouse in study 1, the technological stages of suppressing the animal's life by the conventional method were observed, and no accidental falls of the lambs were observed in the adduction corridor.

Samples collected from lambs slaughtered in the traditional system in households obtained lower average values (2.22 μg/dL) of the cortisol level, compared to the average values of blood samples collected from lambs slaughtered conventionally (4.61 μg/dL) and halal (4.70 μg/dL), a fact that most probably correlates with the way the animals are reared, with the fact that they do not suffer from transport stress and with the fact that the animals are not kept in crowded groups.

Following the statistical analysis of the three batches, analyzed by the ANOVA one-way test it resulted that there were significant differences within the cortisol levels recorded for all the batches ($P<0.05$).

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