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COMPARATIVE STUDY ON THE VARIATION OF THE SERUM CORTISOL LEVEL DEPENDING ON THE CATTLE SLAUGHTERING METHOD

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Abstract

Over time, meat has played an essential role in human evolution and is an important component of a healthy and balanced diet, a fact due to the nutritional richness that varies depending on a number of factors. Pre-slaughter stress is a crucial factor in meat quality and safety. Animals intended for slaughter are stressed by a variety of endogenous and exogenous factors. Slaughter is a complex process, and there is clear evidence in the literature, that pre-slaughter stress is harmful to the meat quality. Therefore, the purpose of stunning is to render animals unconscious during bleeding, without causing pain or stress. In some countries, cattle are slaughtered by a religious method, without stunning, namely the halal slaughter practiced by Muslims and the kosher slaughter practiced by Jews. The study was carried out during 2020-2022, on two batches of conventionally slaughtered cattle (with stunning), on one batch of halal slaughtered cattle (without stunning and on one batch of traditionally slaughtered cattle (without stunning). Within the slaughterhouses, the technological flow of slaughtering cattle was followed and blood samples were collected in order to extract serum and dose cortisol. Cortisol was dosed in a specialized laboratory using 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 batches slaughtered in the traditional halal system, compared to the conventionally slaughtered batches. The values recorded for all four batches, exceed the reference range of 0.47-0.75 µg/dL. Excessive handling of cattle induces their stress, therefore special attention must be paid to the rest period before slaughter, to physiologically rebalance the body, but also to the slaughtering process, to minimize stress levels and ultimately improve meat quality obtained, because it has been shown that there is a direct correlation between the quality of the meat and the way the animals are slaughtered, more precisely with stunning or without stunning.

Key words*: cattle, cortisol, halal, slaughter, stress.*

INTRODUCTION

Meat has played an essential role in human evolution and represent an important component of a balanced and healthy diet, a fact due to its nutritional richness (Williamson et al, 2005; McNeill & Van Elswyk, 2012; Pereira & Vicente, 2012; Petcu, 2013; Predescu et al., 2018; Mihai et al., 2021).

The nutritional composition of meat varies depending on a number of factors, such as: breed, sex, age, diet, fattening status, body weight, animal health, rational feeding, animal movement, but also on the slaughtering method, here thinking to slaughter with stunning (conventional) or slaughter without stunning (traditional, halal, kosher) (Williams, 2007; Banu et al., 2009).

In the living organism, a series of energetic and biochemical transformations occur, which are in close interdependence and which stop with the suppression of the animal's life (Ionescu & Diaconescu, 2010; Mihai et al., 2021).

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).

Acute stress lasts from a few minutes to several hours, and chronic stress persists several hours a day, for weeks or months (Dhabhar, 2002; Ciliberti et al., 2017).

The increasing global commercialization of beef has led to an increase in the number of animals slaughtered (Blokhuis et al., 2008),

resulting in animal welfare issues, related to transport to the slaughterhouse and handling before slaughter have worsened (Miranda de la Lama et al., 2014; Pérez-Linares et al., 2015).
Due to the increasingly frequent change in the mentality of consumers and their desire to consume only safe food that does not have a negative impact on their own health, on the environment, nor on the way animals are reared, cattle welfare has become a social issue and broadened the concept of food quality (Savu et al., 2002; María, 2006; Sepúlveda et al., 2008; Pérez-Linares et al., 2015; Petcu, 2015).
Pre-slaughter stress is a crucial factor in meat production. Animals destined for slaughter are stressed by a variety of endogenous and exogenous factors, which adversely affect complex post-mortem biochemical reactions (Franco et al., 2015). The most common factors are: weather changes, transport conditions, how the animals are handled, the waiting period before slaughter, the stunning procedure and the method of slaughter (Apple, 2005).
The magnitude of any negative effect depends on the type, duration and intensity of the stressors, before the animal is slaughtered, and how susceptible it is to them (Ferguson et al., 2001; Ferguson & Warner, 2009; Pérez-Linares et al., 2015).
These pre-slaughter adverse effects not only have an impact on animal welfare, but also have negative effects on meat quality. Particularly in cattle, there is evidence that pre-slaughter stress is detrimental to meat quality (Ferguson, 2008). These adverse effects on beef quality can lead to Dark Firm Dry (DFD) meat (Franco et al., 2015; Pérez-Linares et al., 2015).
In the period before slaughter, stress and physical activity contribute to the depletion of muscle glycogen and, as a result of this increased pH, the beef changes its appearance, and can be classified as DFD (dark, firm and dry) (Pérez-Linares et al., 2015).
DFD meat is highly susceptible to bacterial problems due to the increased pH ($\geq$ 5.8) (Van de Water et al., 2003). It is difficult to market DFD meat, because the consumer associates its dark colour with improper storage conditions or assumes that the beef is outdated (Mounier et al., 2006).
The slaughter process is quite complex, as it is characterized by several stressful stages, caused by numerous factors. Consequently, the purpose of stunning is to render animals unconscious during bleeding without causing pain or stress. In many countries, however, it is a common practice to slaughter cattle by a religious method, practiced without stunning, and here we refer to the halal slaughter practiced by Muslims and the kosher slaughter practiced by Jews (Önenç & Kaya, 2004; Barrasso, 2021).
Based on available data, it has been shown that almost 26 million Muslims and 1.1 million Jews live in the European Union. Thus, animals slaughtered according with religious rituals are reared in the EU (Europe's Growing Muslim Population, 2017).
During slaughter, animal stress can be physical, psychological, or both physical and psychological (Lawrie, 1966; Barrasso, 2021).
The physiological response to stress involves the secretion of stress hormones such as cortisol and catecholamines (adrenaline and noradrenaline) (Linares et al., 2008; Terlouw et al., 2021).
Cortisol determination is one of the most widely used methods for assessing stress in animals. It can be dosed from blood (serum or plasma), saliva, urine, faeces, milk and hair (Casal et al., 2017).

MATERIALS AND METHODS

The study was conducted in the period 2020-2022 on two batches of conventionally slaughtered cattle (with stunning), on one batch of halal slaughtered cattle (without stunning) and on one batch of traditionally slaughtered cattle (without stunning). Blood samples were collected as follows:
- Batch 1: 15 blood samples collected in November 2020 (cold season), from a batch of 100 cattle, different breeds, slaughtered after stunning, in a slaughterhouse.
- Batch 2: 15 blood samples collected in October 2021, from a batch of 60 cattle, different breeds, slaughtered without stunning in a slaughterhouse, by the halal method, specific to Muslims.
- Batch 3: 15 blood samples collected in August 2022 (warm season), from a batch of 98

cattle, different breeds, slaughtered after stunning, in a slaughterhouse.
- Batch 4: 6 blood samples collected in December 2022 (cold season), from traditionnally slaughtered cattle.
In the slaughterhouses, the technological flow of slaughtering cattle was followed and blood samples were collected.
In the case of **conventional slaughter**, the cattle enter the adduction corridor and are mechanically stunned, using a stun gun with a captive bolt, positioned at the level of the head. Dividing the skull to find the ideal location for penetrating the brain is done imaginary, by drawing two lines starting from the eye to the opposite horn. Immediately after the stunning, hanging on the conveyor line takes place, and the bleeding stage will take place in the shortest possible time.
In the case of **halal slaughter**, the cattle are slaughtered without stunning. Each cattle is placed in the individual rotating containment box and the designated person in the slaughterhouse performs the sectioning of the blood vessels in one movement, using a sharp knife, not before saying *Bismillah*.
In the case of **traditional slaughter**, cattle are slaughtered without stunning.
The present study aims to carry out laboratory analyzes aimed at measuring the level of cortisol in blood samples (Figure 1), 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 labelling and transported immediately to a specialized laboratory, cortisol being dosed by the Immunoenzymatic method with chemiluminescence detection.

Figure 1. Blood samples collected from cattle slaughtered in the slaughterhouse

In the present study, cortisol was assayed from serum (Figure 2) obtained from blood samples collected from the bleeding wound.
To determine these parameters, specialized training and laboratory equipment, as well as specific materials and reagents, are necessary.

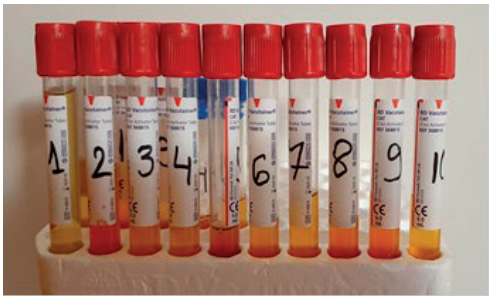

Figure 2. Serum samples used for cortisol dosing

RESULTS AND DISCUSSIONS

It has been shown that there is a direct correlation between the way animals are slaughtered (with or without stunning) and the stress during slaughter, which directly influences the meat quality.
Also, the period of slaughter, in direct correlation with the temperature of the environment, 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).

Results and discussions regarding blood cortisol levels

It has been shown that stress before slaughter has a negative impact on the hormonal system of animals and implicitly on the meat quality (D'Eath et al., 2010; Mihai et al., 2021).
One of the most used methods for assessing stress in animals is the determination of cortisol, because it provides information about the activity of the hypothalamic-pituitary-adrenal axis (Casal et al., 2017; Mihai et al., 2021).
The determination of the cortisol level in the blood samples collected after the slaughter of the cattle, highlighted different values, which exceed the reference interval, established by Jackson and collaborators in 2002, namely 0.47-0.75 μg/dL.

Study 1 - determination of cortisol in blood samples collected from conventionally slaughtered cattle in November 2020 (Conventional 1)

Following the analysis of the cortisol level of the 15 blood samples collected from conventionally slaughtered cattle in November 2020, it was observed that all samples had higher values compared to the reference interval (0.47-0.75 µg/dL).

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

Table 1. Results obtained after cortisol dosing in cattle slaughtered in the conventional system from batch 1 (Conventional 1)

No.	Slaughtering date	Sex	Age	Cortisol level µg/dL
1.	19.11.2020	F	14 years	**1.64**
2.	19.11.2020	F	3 years	**1.61**
3.	19.11.2020	F	4 years	**1.02**
4.	19.11.2020	F	10 years	**3.87**
5.	19.11.2020	F	5 years	**1.10**
6.	19.11.2020	F	4 years	**2.53**
7.	19.11.2020	F	6 years	**4.15**
8.	19.11.2020	F	14 years	**4.98**
9.	19.11.2020	F	3 years	**7.73**
10.	19.11.2020	F	13 years	**3.82**
11.	19.11.2020	F	5 years	**2.92**
12.	19.11.2020	F	13 years	**4.13**
13.	19.11.2020	F	3 years	**4.34**
14.	19.11.2020	F	4 years	**2.15**
15.	19.11.2020	F	10 years	**2.35**

Study 2 - determination of cortisol from blood samples collected from halal slaughtered cattle in October 2021 (Halal)

In October 2021, 15 blood samples collected from cattle slaughtered in a halal system in a slaughterhouse were analyzed in a specialized laboratory. All immunoassay cortisol values were above the reference interval. The lowest value recorded was 1.46 µg/dL, and the highest value 7.57 µg/dL, the accepted reference interval being 0.47-0.75 µg/dL.

Sample number 1 recorded a value 10 times higher compared to the maximum reference value. The results are presented in Table 2.

Table 2. Results obtained after cortisol dosing in cattle slaughtered in the halal system from batch 2 (Halal)

No.	Slaughtering date	Sex	Age	Cortisol level µg/dL
1.	11.10.2021	M	4 years	**7.57**
2.	11.10.2021	F	3 years	**7.34**
3.	11.10.2021	F	4 years	**5.26**
4.	11.10.2021	M	5 years	**2.82**
5.	11.10.2021	M	5 years	**1.46**
6.	11.10.2021	F	9 years	**3.27**
7.	11.10.2021	F	8 years	**6.12**
8.	11.10.2021	F	3 years	**5.20**
9.	11.10.2021	M	9 years	**6.98**
10.	11.10.2021	F	5 years	**7.10**
11.	11.10.2021	F	9 years	**2.27**
12.	11.10.2021	F	8 years	**5.02**
13.	11.10.2021	F	3 years	**5.20**
14.	11.10.2021	M	9 years	**4.68**
15.	11.10.2021	F	5 years	**6.10**

Study 3 - determination of cortisol in blood samples collected from conventionally slaughtered cattle in August 2022 (Conventional 2)

Following the analysis of the cortisol level of the 15 blood samples collected from conventionally slaughtered cattle in August 2022, it was observed that all samples had higher values compared to the reference interval (0.47-0.75 µg/dL). Sample number 9 recorded a value 7 times higher compared to the reference interval, namely 5.55 µg/dL.

It is found that animals slaughtered in the warm season recorded lower cortisol values, which are slightly closer to the reference interval, compared to animals slaughtered in the cold season, which recorded higher values.

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 cattle slaughtered in the conventional system from batch 3 (Conventional 2)

No.	Slaughtering date	Sex	Age	Cortisol level µg/dL
1.	30.08.2022	F	10 years	**1.20**
2.	30.08.2022	F	4 years	**2.22**
3.	30.08.2022	F	5 years	**1.66**
4.	30.08.2022	F	5 years	**1.98**
5.	30.08.2022	F	5 years	**1.07**
6.	30.08.2022	F	12 years	**1.91**
7.	30.08.2022	F	9 years	**3.49**
8.	30.08.2022	F	14 years	**1.17**
9.	30.08.2022	F	8 years	**5.55**
10.	30.08.2022	F	13 years	**2.44**
11.	30.08.2022	F	3 years	**1.15**

No.	Slaughtering date	Sex	Age	Cortisol level µg/dL
12.	30.08.2022	M	3 years	**1.19**
13.	30.08.2022	F	3 years	**1.35**
14.	30.08.2022	F	10 years	**3.23**
15.	30.08.2022	F	5 years	**1.51**

Study 4 - determination of cortisol in blood samples collected from traditionally slaughtered cattle in December 2022 (Traditional)

Following the analysis of the cortisol level of the 6 blood samples collected from traditionally slaughtered cattle in December 2022, it was observed that all samples had higher values compared to the reference interval (0.47-0.75 µg/dL). Sample number 1 recorded 9.10 µg/dL, the value exceeding 12 times the accepted reference maximum value, established by Jackson & Cockcroft. The results obtained after the cortisol dosage from the samples of batch 4 are presented in Table 4.

Table 4. Results obtained following cortisol dosing in cattle slaughtered in the traditional system from batch 4 (Traditional)

No.	Slaughtering date	Sex	Age	Cortisol level µg/dL
1.	14.12.2022	M	8 months	9.10
2.	14.12.2022	M	10 months	6.14
3.	14.12.2022	M	8 months	7.00
4.	14.12.2022	M	8 months	4.30
5.	14.12.2022	F	12 months	4.14
6.	14.12.2022	M	8 months	5.22

Results and discussions regarding the statistical analysis of the data

The results obtained from the summary statistics (mean values, standard deviation, standard error of the mean, median, maximum and minimum values) of serum cortisol samples collected from slaughtered animals are presented in Figure 3.

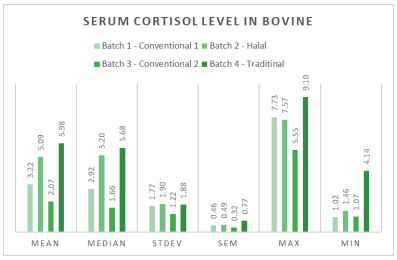

Figure 3. Summary statistics of serum cortisol level in bovine (mean values, median, standard deviation, standard error of the mean, max. and min. values) (µg/dL)

Analysing the four batches statistically, following an ANOVA one-way analysis of variance test, using the GraphPad Prism Statistical Software, it resulted that there were significant differences within the cortisol levels recorded for all the batches ($P<0.05$) (Figure 4).

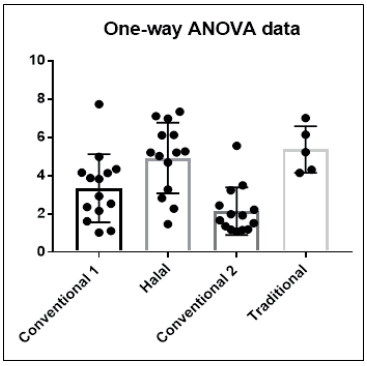

Figure 4. Summary statistics of ANOVA one-way analysis

Comparing the results obtained, it can be observed that higher values of the cortisol level were recorded in the batches slaughtered in the traditional and halal system, compared to the batches slaughtered conventionally.

The first batch was slaughtered in November, the second batch in October, the third batch in August and the fourth batch in December.

The highest cortisol value was recorded in one of the traditionally slaughtered calves.

According to numerous studies, an important factor that influences the stress level of animals is the environment temperature (Guerrini & Bertchinger, 1982; Linares et al., 2008; Deiss et al., 2009; Salaberger, 2016; Mihai et al., 2021).

The values recorded for all four batches exceed the reference interval of 0.47-0.75 µg/dL.

Handling operations before animal slaughter, certainly induce a stress response, affecting the condition of the animal (Śmiecińska et al., 2011). Optimal preparation of animals before slaughter, by ensuring rest immediately after transport, alleviates stress and physiologically balances the organism (Gispert et al., 2000; Fischer, 2001; Śmiecińska et al., 2011; Petcu, 2015).

Recent studies in northwestern Mexico, reported an incidence of DFD meat during the warm season (summer), and concluded that both pre-slaughter and post-slaughter factors contribute

to the occurrence of DFD meat. The resting period before slaughter, the relative humidity of the environment and the time elapsed between slaughters were relevant factors during the pre-slaughter period, while the chilling temperature and the time spent by the carcass in storage areas were found to be important post-slauthering factors (Pérez-Linares et al., 2008; Sotelo-Flores, 2008; Leyva-García et al., 2012; Pérez-Linares et al., 2015).

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 5.

Table 5. Summary statistics of serum cortisol level in bovine - t-test (Student test)

Batches analyzed	P - value	Significantly different (P < 0.05)	Mean ± SEM (Standard error of the mean)
Conventional 1 - Halal	0.0095	Yes	3.223 ± 0.458, n=15 5.093 ± 0.4915, n=15
Conventional 1 - Conventional 2	0.0483	Yes	3.223 ± 0.458, n=15 2.075 ± 0.3151, n=15
Conventional 1 - Traditional	0.005	Yes	3.223 ± 0.458, n=15 5.983 ± 0.7656, n=6
Halal - Conventional 2	< 0.0001	Yes	5.093 ± 0.4915, n=15 2.075 ± 0.3151, n=15
Halal - Traditional	0.3431	No	5.093 ± 0.4915, n=15 5.983 ± 0.7656, n=6
Conventional 2 - Traditional	< 0.0001	Yes	2.075 ± 0.3151, n=15 5.983 ± 0.7656, n=6

Batch 1 slaughtered conventionally in November and batch 2 slaughtered halal obtained statistically significant differences (P < 0.05) (Figure 5).

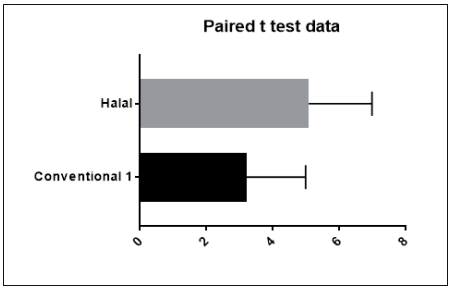

Figure 5. Mean value between batch 1 and batch 2

Batch 1 slaughtered conventionally in November and batch 3 slaughtered conventionally in August obtained statistically significant differences (P < 0.05) (Figure 6). The samples of batch 3 were collected in the warm season (August), compared to the samples of batch 1, which were collected in the cold season (November).

Batch 1 recorded a mean cortisol value of 3.22 µg/dL, a value that is higher compared to the mean cortisol value recorded for batch 3, namely 2.07 µg/dL. Both batches of animals were conventionally slaughtered in the same slaughterhouse, the difference in results correlating, most likely with the season in which these animals were slaughtered.

The time of harvesting samples may be an explanation for the fact that samples collected from animals slaughtered in the summer recorded lower cortisol levels, compared to samples collected from animals slaughtered in the late autumn, a fact that is also explained by Guerrini and Bertchinger in their studies, showing that the lowest values of plasma cortisol were recorded during the exposure of the animals to a warm environment, and the highest values were recorded when the animals were exposed to a cool and humid environment.

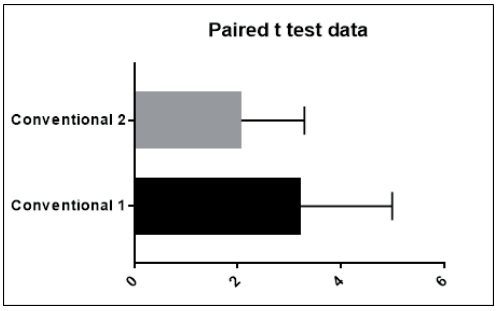

Figure 6. Mean value between batch 1 and batch 3

Batch 1 slaughtered conventionally in November and batch 4 slaughtered traditionally obtained statistically significant differences ($P < 0.05$) (Figure 7).

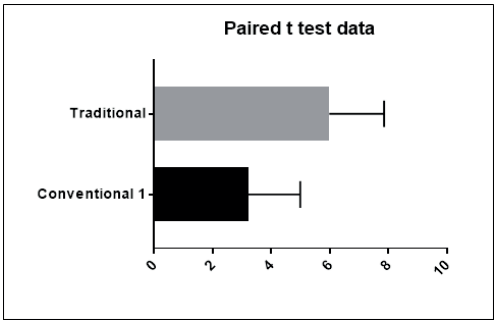

Figure 7. Mean value between batch 1 and batch 4

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

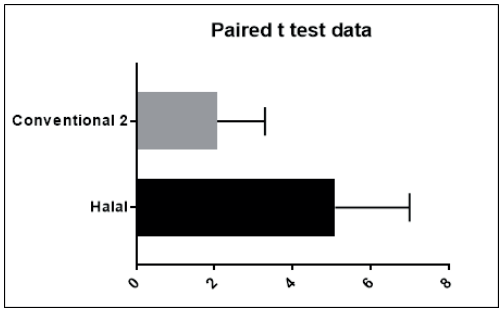

Figure 8. Mean value between batch 2 and batch 3

Batch 2 slaughtered halal and batch 4 slaughtered tradutionally obtained statistically insignificant differences ($P > 0.05$).

Both groups were slaughtered without stunning.

The increased level of cortisol is an indicator of the animals' stress response, resulting from the stimulation of the hypothalamic-pituitary-adrenal axis and the sympathetic and parasympathetic nervous system (Śmiecińska et al., 2011).

The mean cortisol value of batch 2 is 5.09 µg/dL, this batch being represented by cattle slaughtered halal in October.

Batch 4, represented by calves traditionally slaughtered in December, recorded an average value of 5.98 µg/dL (Figure 9).

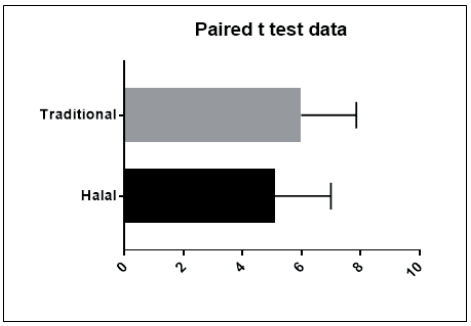

Figure 9. Mean value between batch 2 and batch 4

Batch 3 slaughtered conventionally in August and batch 4 slaughtered tradutionally obtained statistically significant differences ($P < 0.05$) (Figure 10).

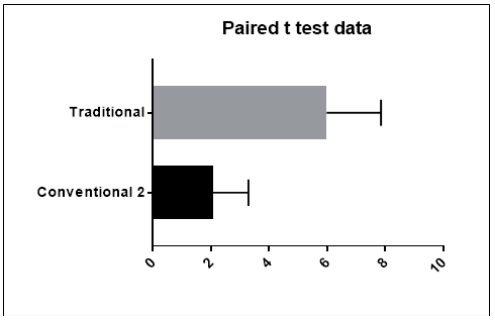

Figure 10. Mean value between batch 3 and batch 4

CONCLUSIONS

In the study slaughterhouses, all stages of the technological flow of slaughtering animals are respected, both for conventionally slaughtered (with stunning) and for traditionally and halal slaughtered (without stunning).

The refusal of the animals to enter the containment box and the accidental fall of the animals on the adduction corridor were not observed.

The method of stunning, practiced in conventional slaughter is mechanical stunning with a captive bolt gun.

Excessive handling of cattle induces their stress, therefore special attention must be paid to the rest period before slaughter, to physiologically balance the body, but also to the slaughtering process, to minimize stress levels and ultimately improve meat quality obtained.

Samples collected from cattle slaughtered in the conventional system obtained a lower average value of cortisol compared to the average value obtained for blood samples collected from traditionally and halal slaughtered cattle, a fact that most likely correlates with the way the animals are reared, with the individual reactivity of each animal, with the transport stress and with the season in which the animals were slaughtered.

Following the statistical analysis of the four 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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