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## Stress Assessment by Measuring Cortisol Levels in Cattle at a Slaughterhouse in Algeria

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### Abstract:

The pre-slaughter period includes a series of essential steps (loading, transport, unloading, lairage, and slaughter) likely to induce physical and/or psychological stress in cattle. Blood cortisol concentration is a reliable indicator of stress, particularly psychological, as it reacts more slowly to stress factors and recovers less quickly than other markers. This study aims to evaluate the effects of animal-related, environmental, and slaughter procedure factors on plasma cortisol levels in cattle at the time of bleeding. A total of 160 cattle of different breeds and ages were observed. Collected data included origin (farm or market), breed, age, transport duration, lairage time, and waiting time in the slaughter room. Blood samples were collected at bleeding, and Cortisol levels were measured using the VIDAS automated system, which uses the ELFA (Enzyme-Linked Fluorescent Assay) method, combining ELISA (Enzyme-Linked Immunosorbent Assay) with final fluorescence reading in blue light. The mean concentration observed was 35.26 ng/mL. Significant differences were noted between breeds, lairage durations, and waiting times in the slaughter room. However, age, origin, and season did not show significant differences. The results indicate variability in stress reactivity depending on the breed. **Keywords:** Stress, Cattle, Cortisol, Slaughterhouse, Algeria.

## **I. Introduction:**

The pre-slaughter period begins with the preparation of the animal at the farm and ends with its slaughter (**Bourguet., 2010b**). During this period, animals are exposed to a series of factors and procedures that can induce a state of stress. These factors can be of physical origin—such as fasting, fatigue, and pain—related to food deprivation, water restriction, truck movements, shocks caused by loss of balance, or blows received from other animals or handlers (**Pérez-Linares et al., 2015; Terlouw et al., 2015; Terlouw., 2002; Knowles., 1999; Bourguet., 2010b**). Alternatively, stress can be of psychological origin, resulting from separation, mixing with unfamiliar animals, exposure to novelty, or human contact (**Grandin., 1997; Knowles., 1999; Terlouw et al., 2007; Terlouw et al., 2012; Bourguet., 2010b**). Indeed, numerous studies conducted in various countries worldwide have aimed to understand the causes and consequences of slaughter-related stress by evaluating behavioral and physiological responses. Some of these studies have been carried out under controlled conditions, while others have been conducted in practical settings, particularly in developed countries. This research has led to improvements in slaughter conditions, thereby enhancing both animal welfare and meat quality. However, developing countries like Algeria, remain uninformed about the behavioral responses of locally raised cattle breeds to stress, as well as their reactions to the procedures applied in slaughterhouses (**Terlouwet al., 2011; Bonou et al., 2017; Njisane&Muchenje., 2017**).

Both in controlled and practical conditions it is essential to understand how animals perceive the various aspects of slaughter procedures and how they respond under real-world conditions. Therefore, on one hand, it is crucial to clarify the concept of stress, its potential causes, and its effects to mitigate stress effects on animal welfare and meat quality, particularly in locally raised livestock in developing countries. On the other hand, conducting pre-slaughter research under practical conditions is essential to establish the best practices for animal welfare during slaughter and to enhance meat quality (**Terlouwet al., 2011; Bonou et al., 2017; Njisane&Muchenje., 2017**).

This study aims to elucidate the effects of bovine characteristics (age, breed), environmental factors (season, origin of animals—farm or market), waiting duration at the slaughterhouse, and time spent in the slaughtering area on cortisol level and the stress levels of cattle slaughtered in a private slaughterhouse in Algeria.

## **II. Materials and Methods:**

This study was conducted under field conditions during routine slaughtering, without interfering with the slaughtering process, on cattle destined for slaughter at a private slaughterhouse of Frères Bouaouadja in Ouled Saber, Sétif province, Algeria. The selection of this slaughterhouse was based on approval from the supervising veterinary inspector, the slaughterhouse owner, the cattle owners, the Directorate of Agricultural Services, and the veterinary inspector of Sétif province to collect blood samples. The slaughterhouse complied with national regulations on meat inspection, monitoring, and control, as well as all meat product standards. This study was carried out during the summer, from June 1, 2023, to June 30, 2023, and in the autumn, from November 1, 2023, to November 15, 2023, with a total duration of one and a half months.

### **II.1. Slaughterhouse:**

The slaughterhouse is located in the commune of Ouled Saber, 5 km from the capital of Sétif province, within the industrial zone. On average, the slaughterhouse processes thirty cattle per day, with a capacity of up to sixty cattle per day. Unloading is carried out at the designated storage area dock.

### **II.2. Animals:**

A total of 160 healthy males of different breeds and ages were included in the study. These animals belonged to 12 butchers and were transported either from farms or livestock markets. The farm-sourced cattle had various origins: some were fattened by their owners, others were purchased from different farms either in advance or the day before slaughter, while some were acquired from livestock markets 2 to 6 days prior to slaughter. Cattle from farms were transported either individually or in groups of 2 to 10 animals. In contrast, those from livestock markets were transported in groups of more than 10 animals. Most of the studied cattle were unloaded immediately upon arrival at the slaughterhouse, directly onto the unloading dock. However, some butchers preferred to keep their animals inside the trucks until their turn, while others arrived the day before slaughter. Observations were conducted by two individuals, one stationed outside and the other inside the slaughtering hall. The monitoring process included recording the time of arrival, transport duration, animal origin, breed, age, time of entry into the slaughtering hall, and slaughter time. Breed and age determination were based on external morphology and dentition, respectively. Transport

duration and other missing information were recorded based on declarations from the slaughterhouse owner, butchers, and slaughterhouse workers. Cattle were numbered twice: upon owner approval and arrival at the slaughterhouse to record general animal information, and during slaughter according to the slaughtering sequence. The latter numbering was used for individual cattle observation records.

### **II.3. Slaughter Conditions:**

At the slaughterhouse, cattle are slaughtered promptly upon arrival unless a large number of animals are present. In such cases, they are held in the holding pen or kept in the truck until their scheduled bleeding. The entry of animals into the slaughtering hall occurs in groups of 5 to 10 cattle, except when the number is insufficient. Once inside, the animals are placed in lateral recumbency and subsequently bled by severing both jugulars veins, the esophagus, and the trachea using a well-sharpened knife of appropriate size for the cattle.

### **II.4. Blood samples and storage conditions:**

A blood sample was collected during exsanguination into heparinized tubes (AFCO, lithium heparin, AF20011B, 4 mL), each labeled with the individual animal identification number. This was done for all 160 observed cattle, in accordance with the protocol described by Terlouw et al. (2011). One tube was collected per animal. Immediately after collection, the samples were centrifuged at 2000 rpm for 10 minutes using a semi-automatic centrifuge (Model 800D, capacity 20ml/6, Chinamax speed 4000 rpm, max RCF 1790\*g, made in China). The plasma was then extracted into dry tubes and stored in a cooler filled with ice. After 4 hours, the samples were transported to the laboratory (private medical analysis laboratory Dr. Osmani. M, located in the municipality of AinArnat, 10 km from the slaughterhouse), and then kept frozen until the following day for cortisol measurement.

### **II.5. Cortisol Assay:**

Cortisol levels were measured using the VIDAS® automated immunoassay system. The selection of this system was based on a validation test conducted on bovine serum, as reported by Proverbio et al., 2013.

- **Dosage Technique:**

The VIDAS Cortisol S test is an automated assay performed on the VIDAS system, allowing for the direct quantitative measurement of cortisol in serum or plasma.

Plasma cortisol concentrations were determined using the Enzyme-Linked Fluorescent Assay (ELFA) technique, which is based on the principles of the Enzyme-Linked Immunosorbent Assay (ELISA). The analysis was performed using the automated VIDAS® system, following the manufacturer's instructions. Prior to the initial assay, the analyzer was thoroughly cleaned, calibrated, and operated according to standard operating procedures. The methodology followed was consistent with that described by Proverbio et al. (2013).

First, the necessary reagents are removed from the refrigerator according to the number of samples. A "CORS" cartridge and a "CORS" cone are used for each sample. The required cartridges and cones are then placed in the first compartment of the system. Next, the sample tube is taken, and using a disposable pipette tip, 100 µL of plasma is aspirated and dispensed into the designated sample well. This process is repeated until all test samples are placed in the wells of the compartment cartridges. Once all samples are prepared, the compartment is closed, and the analyzer is started. The system runs for 38 minutes to generate the results. At the end, the displayed results are retrieved and recorded in a pre-prepared file for cortisol values. The results are then copied into the individual records of the corresponding animals.

## **II.6. Statistical Analysis:**

The collected data were subjected to various types of statistical analyses based on the study's objectives. Descriptive analyses were presented in the form of mean plasma cortisol concentrations. Mean comparisons were performed after conducting a normality test. The Student's t-test was applied when comparing two means, while Fisher's ANOVA was used for multiple mean comparisons if data normality was confirmed. Otherwise, these tests were replaced by non-parametric alternatives: the Mann-Whitney test for two means and the Kruskal-Wallis test for multiple means. Additionally, ONE-Way ANOVA and post hoc tests were employed. All analyses were conducted using SPSS 26 software.

### III. Results:

#### III.1. Distribution of Plasma Cortisol Levels in the Sample:

**Table 1:**Distribution of Plasma Cortisol Levels

Colonne1	Minimum	Percentile 05	Percentile 25	Mean	Median	Standard Deviation	Percentile 75	Percentile 95	Maximum
Cortisols (ng/ml)	2.4	3.89	22.29	35.26	35.4	18.83	47.6	65.7	117.9

ng/ml: unit of measurement of plasma cortisol

Table 1 represents the distribution of plasma cortisol levels during the study period. The minimum value is 2.4 ng/ml, while the maximum is 117.9 ng/ml. The majority of values range between 3.89 and 65.7 ng/ml, with an average concentration of 35.26 ng/ml.

#### III.2. Study of the Effect of Animal-Related Factors, Environmental Factors, and Slaughter Conditions on Cattle Stress:

**Table 2:** Effect of Animal-Related Factors, Environmental Factors, and Slaughter Conditions on Cattle Stress.

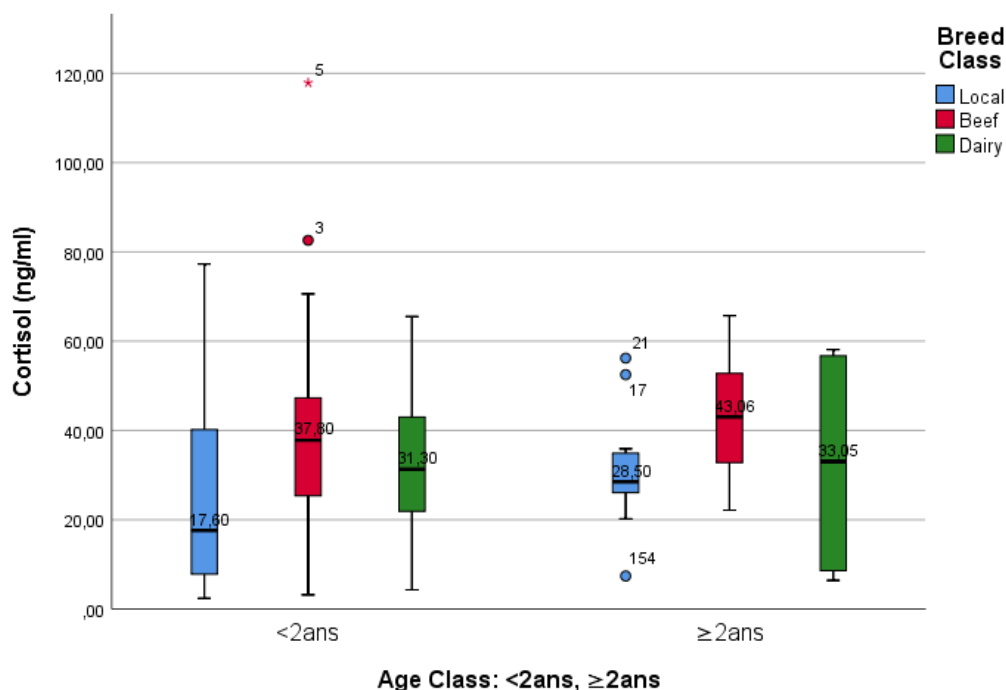
		Number	Mean Cortisol (ng/ml)	Sig
<b>Breed</b>	Local	32	27,85	P< 0,05
	Beef	102	38,29	
	Dairy	26	32,58	
<b>Age</b>	<2 years	127	34,59	0,383
	≥2 years	33	37,81	
<b>Season</b>	Summer	111	34,93	0,741
	Autumn	49	36,01	
<b>Origin</b>	Farm	111	36,30	0,297
	LivestockMarket	49	32,92	
<b>Transport Times</b>	≤1 Hour	75	36,02	0,634
	>1 Hour	85	34,59	
<b>Waiting at slaughter House</b>	<30mn	39	41,62	P< 0,05
	30-60mn	49	37,61	
	61-180mn	30	24,79	
	>180mn	42	33,84	
<b>Waiting for Slaughter</b>	≤5mn	81	29,65	P< 0,001
	6-10mn	40	40,40	
	>10mn	39	41,79	

mn: minute unit of time measurement, Sig : significance level, ng/ml : unit of measurement of plasma cortisol

**Table 2** presents the effect of breed, age, season, origin, transport duration, holding time, and waiting time in the slaughter room on plasma cortisol levels. The cortisol levels were similar in summer and autumn, as well as between animals younger than two years and those aged two years or older. Likewise, no significant difference was observed between animals sourced from the market and those from farms. Meanwhile, we observed that the beef breed (38.29 ng/ml) exhibited a higher plasma cortisol level compared to the local breed and the dairy breed (27.85 ng/ml and 32.58 ng/ml, respectively). Furthermore, our results indicate that cattle held in waiting for 61 to 180 minutes had a lower cortisol level (24.79 ng/ml) compared to those slaughtered within 30 minutes (41.62 ng/ml), those held for 30 to 60 minutes (37.61 ng/ml), and those kept for more than 180 minutes (33.84 ng/ml).

### III.3. Reactivity of the Different Cattle Breeds:

#### III.3.1. According to Their Age:

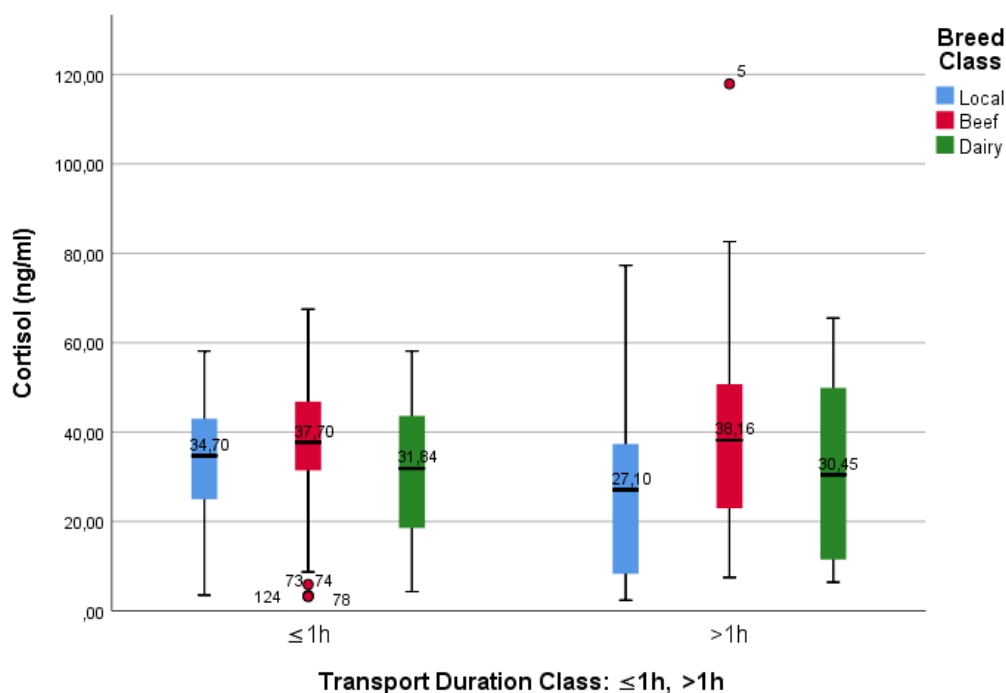


**Figure 1:** Cortisol Levels of the Studied Breeds According to Their Age

The results presented in Figure 1 show that the local breed aged 2 years or older (28.50 ng/ml) is more reactive than those younger than 2 years (17.60 ng/ml). However, we observe only a

slight difference in cortisol levels between the two age categories in both the beef and dairy breeds. Additionally, our results indicate that the beef breed is more reactive (37.80 ng/ml, 43.06 ng/ml) compared to the local and dairy breeds in both age categories (17.60 ng/ml, 31.30 ng/ml) and (28.50 ng/ml, 33.05 ng/ml), respectively.

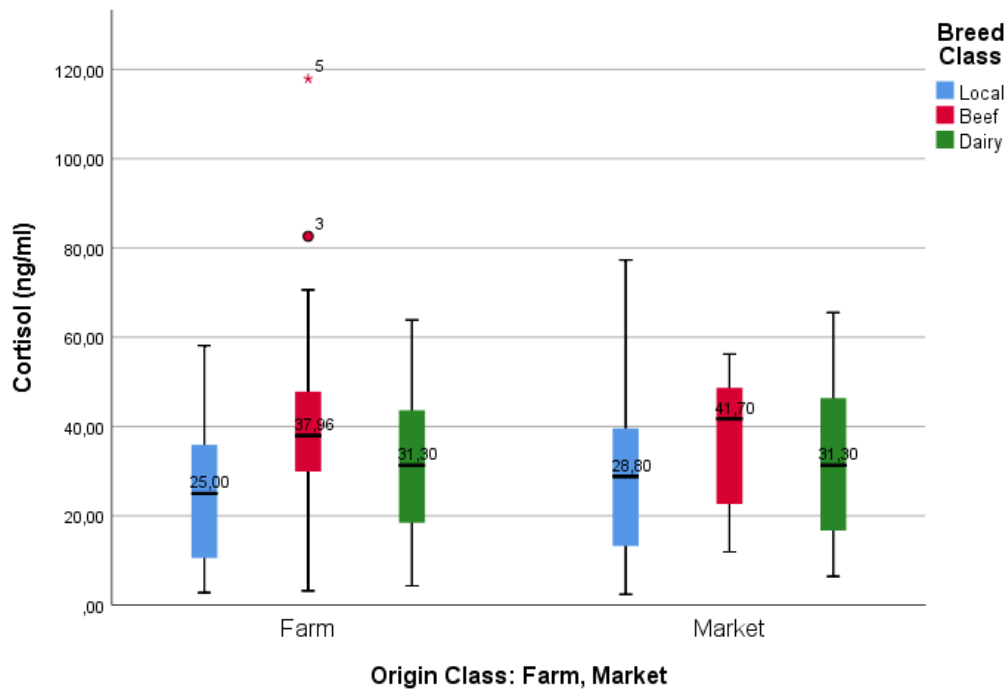
### III.3.2. Reactivity of the Different Studied Cattle Breeds According to The Transport Duration



**Figure 2: Reactivity of Different Cattle Breeds According to Transport Duration**

Our results show that the cortisol level in the local breed transported for more than one hour is lower than in those transported for one hour or less (34.70 ng/ml vs. 27.10 ng/ml, respectively). In contrast, the beef and dairy breeds exhibited only slight differences between the two transport duration categories. However, we observed that the beef breed was more reactive (37.70 ng/ml, 38.16 ng/ml) in both transport duration categories compared to the local and dairy breeds (34.70 ng/ml, 27.10 ng/ml, 31.84 ng/ml, and 30.45 ng/ml, respectively).

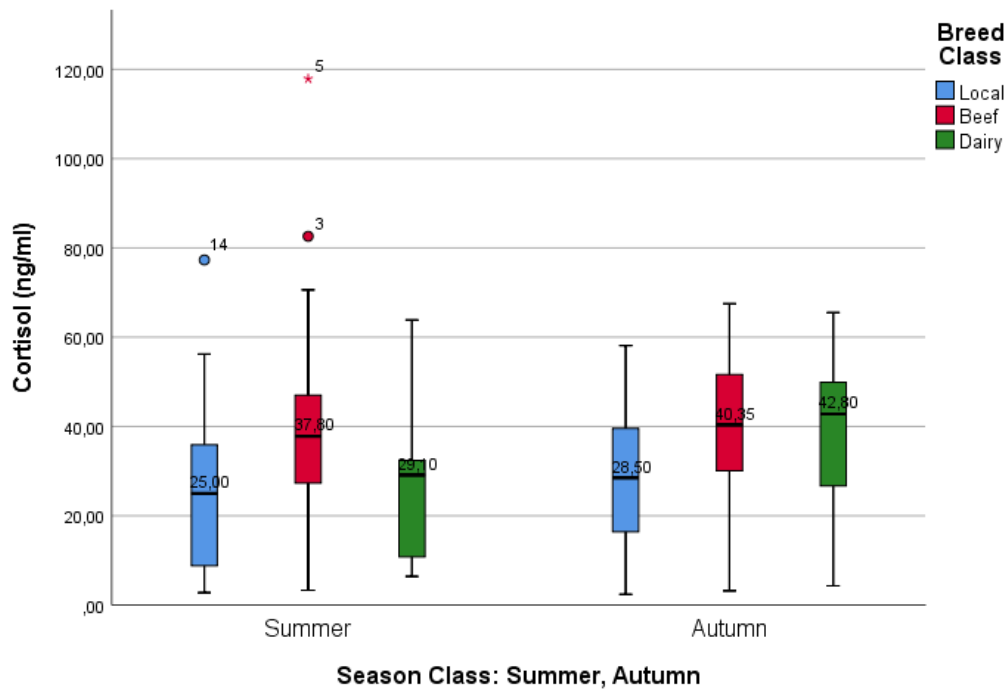
### III.3.3. Reactivity of Different Cattle Breeds Studied According to Their Origin



**Figure 3: Cortisol Levels of Studied Breeds According to Their Origin**

The results presented in Figure 3 show a slight difference in cortisol levels within the same breed between cattle originating from farms and those from markets. Our findings also indicate that the beef breed exhibits higher cortisol levels (37.96 ng/ml, 41.70 ng/ml) compared to the local and dairy breeds in both origin categories (25.00 ng/ml, 28.80 ng/ml, 31.30 ng/ml, 31.30 ng/ml, respectively).

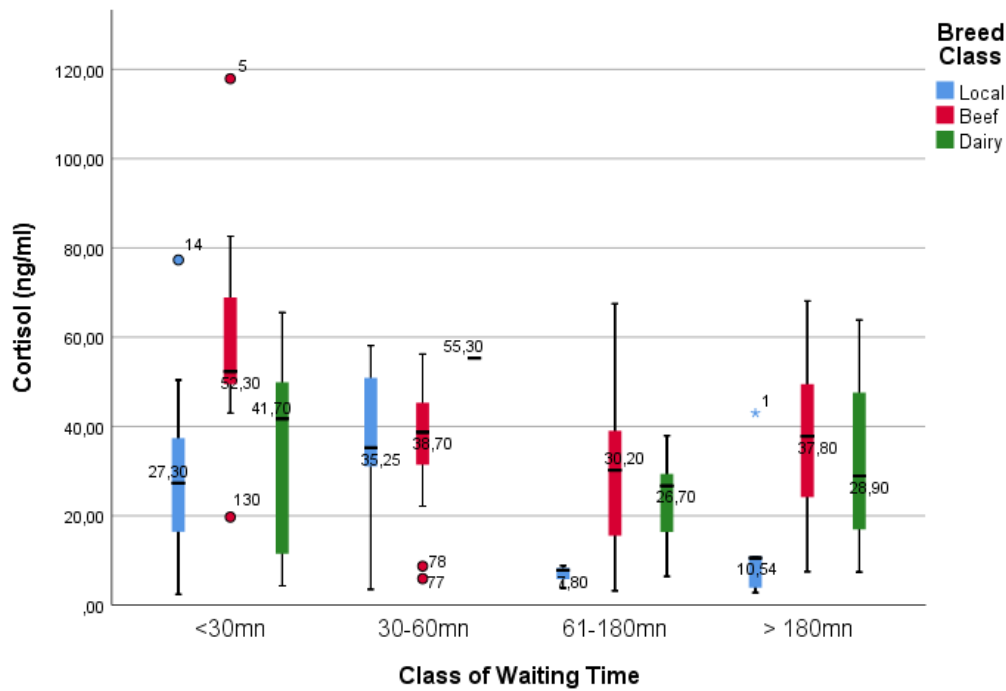
**III.3.4. Reactivity of Different Studied Cattle Breeds According to the Season**



**Figure 4: Cortisol Levels of Studied Breeds According to the Season**

Figure 4 shows that the plasma cortisol level of dairy breed cattle measured in autumn (42.80 ng/ml) is higher than that of the same breed measured in summer (29.10 ng/ml). In contrast, the plasma cortisol level of local and beef breed cattle (28.50 ng/ml, 40.35 ng/ml) measured in autumn is slightly higher than that of the same breeds measured in summer (25.0 ng/ml, 37.80 ng/ml), respectively.

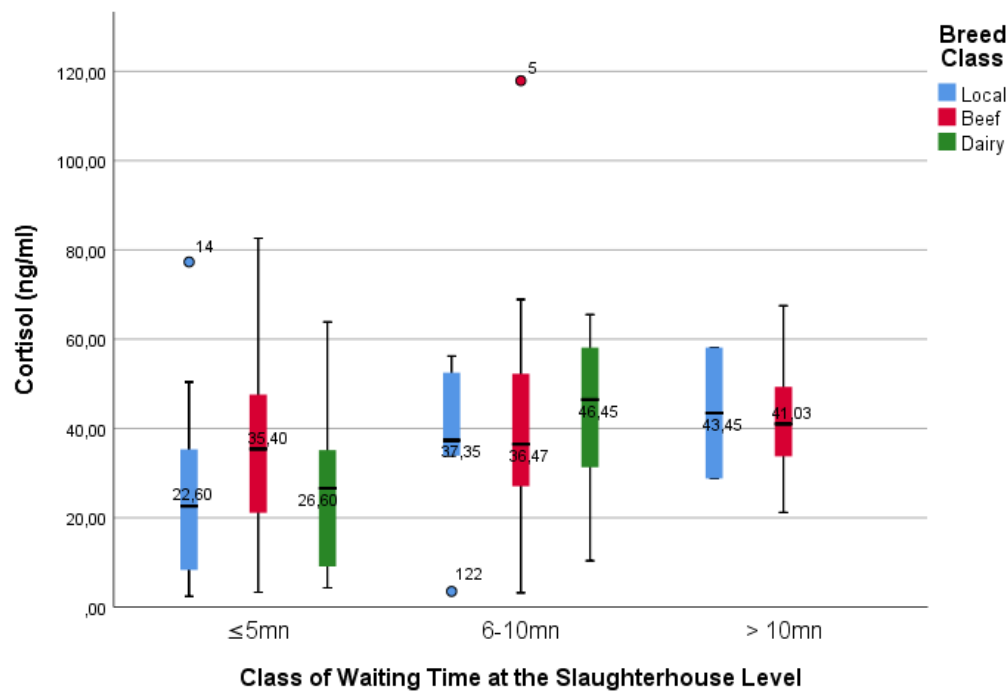
**III.3.5. Reactivity of Different Cattle Breeds According to the Holding Time:**



**Figure 5: Stress Level of Studied Breeds According to Holding Time**

The results presented in Figure 5 show that plasma cortisol levels in the studied breeds decrease with increasing holding time up to 61–180 minutes, then increase in cattle held for more than 180 minutes. We also observe that cortisol levels in beef and dairy cattle are higher (52.30 ng/ml, 38.70 ng/ml, 30.20 ng/ml, 37.80 ng/ml) and (41.70 ng/ml, 26.70 ng/ml, 28.90 ng/ml) compared to local breeds (27.30 ng/ml, 35.25 ng/ml, 4.80 ng/ml, 10.54 ng/ml), respectively.

**III.3.6. Reactivity of the Different Studied Cattle Breeds According to the Waiting Time in the Slaughter Hall**



**Figure 6: Stress Level of Studied Breeds According to the Waiting Time in the Slaughter Hall**

The results presented in Figure 6 show that plasma cortisol levels in the studied cattle breeds increase with longer waiting times in the slaughter hall. However, this increase is more pronounced in local and dairy breeds (22.60 ng/ml, 37.35 ng/ml, 43.45 ng/ml) and (26.60 ng/ml, 46.45 ng/ml), respectively, whereas it is less significant in beef breeds (35.40 ng/ml, 36.47 ng/ml, 41.03 ng/ml).

#### IV. Discussion:

Given that the pre-slaughter conditions to which cattle are subjected induce a state of stress, which can be of physical and/or psychological origin (Knowles., 1999; Bourguet., 2010b), it is crucial to assess its impact, especially in countries where such information are scarce. Psychological stress is primarily linked to fear, induced by changes in environment, human contact, and handling. Fear is considered a major stress factor, as it depends on how the animal perceives the situation it is exposed to, leading to the secretion of cortisol, the stress hormone. The secretion of cortisol is influenced by genetics and the animal's previous experience (Grandin., 1997), as well as by the nature, duration, and intensity of the stress

factors (Terlouwet al., 2012; Loudon et al., 2019). Variations in cortisol levels have been observed in numerous practical and experimental studies (Bourguet., 2010b).

The average cortisol concentration measured in this study was **35.26 ng/mL** (Table 1), which was higher than that reported by **Ewbank et al. (1992)**, where the average blood cortisol concentration in well-handled slaughter cattle was approximately **21.26 ng/mL**. Thus, the cortisol concentration in our study was higher than that reported by **Tume and Shaw. (1992)** in beef cattle slaughtered in a research abattoir, where it reached approximately 15ng/mL. However, it remained lower than the concentration measured in cattle slaughtered in a commercial abattoir, reported by the same authors at 45ng/mL. The elevated cortisol levels observed in this study are likely due to animal stress. We assume that the conditions to which the cattle were subjected induced a stressful state, related to multiple environmental changes (as the cattle originated from markets), loading procedures, slaughter procedures (waiting time, holding time in the slaughter hall), and handling at the slaughterhouse. Additionally, this increase in cortisol levels could result from an interaction between animal-related factors and environmental factors.

#### **IV.1. Effect of Cattle-Related Factors on Plasma Cortisol Levels**

##### **IV.1.1. Breed**

Our results indicate different stress levels among the three cattle breeds studied. Post hoc analysis reveals a statistically significant difference between the beef breed and the local breed (Table 2). The differences in plasma cortisol levels are likely due to the heightened fear response observed in beef cattle compared to the local breed, possibly triggered by the slaughterhouse environment, the presence of humans, or unfamiliar conspecifics.

Additionally, this increased sensitivity may be linked to genetic heritage or rearing conditions, which shape the animals' prior experiences. In general, beef breeds are imported and raised in fattening systems, where their interactions are mostly limited to farm owners and familiar conspecifics. When these animals are transferred to the slaughterhouse, they experience sudden environmental changes, which may induce fear and stress. Our results are consistent with those of **Bourguet (2010b)**, who reported that Blonde d'Aquitaine young bulls are more reactive to isolation and novelty than Angus bulls, suggesting that early rearing conditions play a role in the reactivity of young bulls. Similarly, **Boissy and Le Neindre (1990)** found

that Aubracheifers exhibit more pronounced behavioral responses to isolation compared to Friesians. Furthermore, **Salhi et al. (2019)** observed that local cattle breeds exhibit higher cortisol levels than imported breeds. **Averos et al. (2007)** suggested that differences between pig breeds may be attributed to rearing conditions and the degree of habituation of abreed compared to another. However, **Mormède et al. (2004)**, in a study on pig breeds, demonstrated that genetic background plays a role in the variation of activity and reactivity of neuroendocrine stress response systems.

#### **IV.1.2. Age:**

The results presented in Table 2 indicate that age has no significant effect on plasma cortisol levels. Despite observable differences between cattle aged less than 2 years and those older than 2 years, this variation is not statistically significant. We suggest that this difference is likely due to the interaction of other factors, such as breed and/or the nature and intensity of stressors. However, the analysis of cattle responses based on age and breed (**Figure 1**) reveals that age does not have a significant impact, despite notable differences between the two age categories within the same breed. These differences, however, remain statistically non-significant. Our results do not align with those of **Gebresenbet et al., (2012)**, who reported that calves exhibited higher plasma cortisol levels than cows and bulls after a 2-hour transport period. The authors suggest that calves are more sensitive to environmental novelty, including the transport truck and separation from their conspecifics. **Thus, Bueno et al. (2003) showed that calves weaned at 150 days did not exhibit significant differences in cortisol concentrations compared to non-weaned calves, unlike older calves. The authors suggested that the latter are more capable of initiating short-term stress response mechanisms.**

#### **IV.2. Effect of Environment on Plasma Cortisol Levels**

Our results also indicate that the environment does not have a significant effect on cortisol level variations, as represented by season and animal origin. Despite the lack of statistical significance, we observed that cortisol levels were higher in animals slaughtered in autumn compared to those slaughtered in summer (**Table 2**). We suggest that this may be due to the cold climate, which increases energy demand to maintain body temperature. However, the lack of statistical significance is likely due to interactions with other factors such as breed, lairage duration, and waiting time in the slaughter hall. Our findings do not align with those of **Averos et al., (2007)**, in pigs, or **Baldwin and Stephens.,(1973); Gispert et al., (2000)**.

These authors suggest that cold climate conditions and muscular effort are responsible for seasonal differences observed between winter and summer.

Furthermore, the study of cattle breed reactivity to climatic conditions(**Figure 4**) reveals that the dairy breed is more sensitive to cold climates compared to local and beef breeds. This is likely due to the increased energy demand required for thermoregulation during transport in cold weather, as well as muscle activity caused by truck movements or the effect of isolation (some of these cattle were transported alone). Additionally, cortisol levels were lower in animals from the market compared to those from farms. We suggest that this is because most farm-origin animals are located near the slaughterhouse and are likely slaughtered immediately upon arrival. Moreover, on market days, a large number of animals arrive at the slaughterhouse, and bleeding occurs in order of arrival, allowing animals to recover, which may lead to a decrease in cortisol levels. Alternatively, cortisol levels in market-origin animals might already be declining due to previous exposure to multiple stressors, including environmental changes, transportation twice (from farm to market, then to slaughterhouse), and mixing with unfamiliar animals. These factors may trigger a significant cortisol release, which, through negative feedback mechanisms, could inhibit further cortisol secretion, leading to its reduction.

However, the analysis of cattle breed sensitivity to origin-related stress(**Figure 3**) indicates that beef cattle were the most sensitive, compared to both local and dairy breeds, across both sources of origin. Nevertheless, origin did not have a statistically significant effect on the studied breeds, despite notable differences observed within beef and local breeds between animals sourced from farms and markets. **Our results, consistent with those of Blanco et al. (2009), indicate that breed influenced cortisol concentrations at certain time points, notably at 6 and 168 hours, where Pirenaica calves exhibited higher levels than Parda de Montaña calves.**

### **IV.3. Slaughter Conditions:**

#### **IV.3.1. Effect of Transport on Plasma Cortisol Levels:**

It is well established that transport induces variations in cortisol levels (**Brule., 2004**). Studies have also confirmed that certain factors contribute to stress, including loading, the transport itself, unloading, road conditions, and pre-slaughter procedures (**Terlowet al., 2007**). The

question arises: can cortisol level variations induced by transport still be detected at the time of bleeding?

Our results indicate that transport does not have a significant effect on cortisol level variations, despite observed differences between animals transported for less than one hour and those transported for more than one hour. This discrepancy is likely due to the fact that some cattle are slaughtered immediately upon arrival after transport duration of less than 30 minutes. However, the observed differences are not statistically significant. We suggest that this could be attributed to voluntary or involuntary rest periods imposed on animals transported for more than one hour, possible misreporting by livestock owners, or interactions with other factors present at the slaughterhouse. Either the animal enters an adaptation phase, leading to a decrease in cortisol levels. Our results do not align with those reported by **Bradshaw et al., (1996)**, who observed an increase in cortisol levels 30 minutes after loading, followed by a gradual decrease after 4.5 hours of transport. **Averos et al., (2007)**, in a study on pigs, suggested that short journeys do not allow for complete recovery from the initial stress caused by loading, which is known to have a negative impact on animal welfare. **Thus, Śmiecińska et al. (2011)** reported a significant increase in cortisol concentrations in blood samples collected after transport (during bleeding), compared to those collected before transport. The authors suggest that the pre-slaughter handling procedures induced a marked stress response in the animals studied. **Salhi et al., (2019)**, suggested that stress in cattle is mainly caused by the confrontation with conspecifics and the absence of a waiting area at the slaughterhouse.

#### **IV.3.2 Effect of Waiting Time on Plasma Cortisol Levels:**

The results presented in **Table 2** show that waiting time has a statistically significant effect ( $p < 0.05$ ) on plasma cortisol levels. However, waiting in the slaughterhouse holding area may allow cattle to recover from the exertion and stress of unloading. Nevertheless, the overall environment inside the holding area and the waiting duration can influence animal agitation (**Terlowet al., 2007; Gigena et al., 2021**). Indeed, the difference in plasma cortisol concentration observed in our study is likely due to the fact that, for cattle with a waiting time of less than thirty minutes and those held for 30–60 minutes, the duration was insufficient for recovery from the adverse conditions to which they were exposed (new environment, unfamiliar conspecifics, poor handling, and being left inside the truck). This could potentially

increase aggressiveness and the level of fear. However, a waiting time of more than 180 minutes appears to be stressful, as cortisol levels increased in cattle subjected to this duration compared to those held for 61–180 minutes. This is likely due to extended waiting times with feed deprivation (some cattle were held for more than 8 hours). In contrast, the 61–180 minute waiting time appears to be adequate in our study. Our results are similar to those found by **Lensink et al. (2001)**, who reported that in calves, cortisol concentrations returned to pre-transport levels after 2 hours of waiting in the lairage. Other studies on pigs by **Averos et al. (2007)** showed that cortisol levels at the time of stunning were lower than those recorded at unloading after 6 hours of rest in the lairage, following a 13-hour transport. Additionally, our findings (concerning a waiting time of more than 180 minutes) are consistent with those of **Bourguet et al. (2010a)**, who suggested that prolonged waiting times combined with feed deprivation increase serum cortisol levels in cattle.

The study of the reactivity of beef, dairy, and local breeds to waiting time (**Figure 5**) reveals that the local breed recovers more quickly than the beef and dairy breeds. Our results indicate that the beef breed recovers more slowly, and the waiting times appear to be insufficient for the recovery of this breed, as well as for the dairy breed. The low plasma cortisol levels observed in cattle held for 61-180 minutes are likely due to the cortisol levels of the local breed. **Zavy et al. (1992)** reported that calves with a *Bos indicus* (Brahman) genetic component exhibited significantly higher baseline cortisol concentrations than *Bos taurus* calves, suggesting a greater stress reactivity or increased sensitivity to venipuncture.

#### **IV.3.3. Effect of Waiting Time in the Slaughtering Room on Plasma Cortisol Levels:**

Our results show that the waiting time in the slaughtering room has a statistically significant effect ( $p < 0.001$ ) on plasma cortisol levels. This difference in cortisol concentration is likely due to poor handling or the presence of blood odor in the slaughtering room. Similar findings by **Boissy et al. (1998)** indicate that heifers perceive the increased stress of their conspecifics and consequently become more fearful. The authors also suggest that this perception is at least partially mediated by olfactory signals in the urine. Thus, our results are probably due to the intense fear experienced by the cattle, linked to the perception of danger in response to the desperate situation they face. As presented in Table 2, cortisol levels increase with longer waiting times in the slaughtering room. This finding can be explained by the high sensitivity

of the corticotropic axis to environmental stimuli, even minor ones, and the rapid saturation of the cortisol response as the intensity of stimulation increases (**Mormède., 1995**).

Our results are consistent with those of **Grabovsky et al. (2019)**, who observed significantly higher cortisol levels in rats that were removed from their cages last. The authors suggest that animals experience heightened stress, particularly before slaughter. Similarly, **Bourguet et al. (2010a)** reported that cows subjected to additional stress exhibited higher serum cortisol levels than those experiencing limited stress.

The study on breed reactivity, presented in **Figure 6**, shows that the local breed and the dairy breed are more reactive to waiting time in the slaughtering room compared to the beef breed. This is likely due to the fact that the local and dairy breeds are more sensitive to the smell of blood, the environment, and the situation they are subjected to (slaughtering room, handling, and the slaughter of their conspecifics). **Our results are consistent with those reported by Cochram and Corley. (1991)**, who found that plasma cortisol concentration at the time of slaughter was positively correlated with the time spent in lairage and in the pre-stunning pen in beef cattle at a commercial abattoir.

## V. Conclusion

The current study demonstrates that breed, prior experience, and farming methods play a crucial role in the variation of cortisol levels during the pre-slaughter period. Additionally, our findings reveal that the slaughterhouse environment is highly stressful for beef breeds, as their cortisol levels do not decrease across different waiting times. However, the local breed is highly sensitive to handling and the smell of blood, whereas the dairy breed is particularly sensitive to cold, handling, and the smell of blood. In our study, a waiting time of less than one hour is insufficient for bovine recovery, whereas waiting times exceeding three hours and, more critically, over eight hours are stressful. Furthermore, the waiting period in the slaughtering room is identified as a highly stressful factor.

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