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# INDICATORS OF PRE-SLAUGHTER STRESS IN PIGS AND THEIR IMPACT ON MEAT QUALITY

**Vlad Nicolae ARSENOAIA[1] and Răzvan Nicolae MĂLĂNCUȘ[2*]**

[1]Department of Pedotehnics, Faculty of Agriculture, Iasi University of Life Sciences, 3, Mihail Sadoveanu Alley, 700490, Iasi, Romania; e-mail: vnarsenoaia@uaiasi.ro
[2]Department of Physiology and Pathophysiology, Faculty of Veterinary Medicine, Iasi University of Life Sciences, 8, Mihail Sadoveanu Alley, 700489, Iasi, Romania

*Correspondence: razvanmalancus@uaiasi.ro

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**ABSTRACT**. The pre-slaughter handling and transport of pigs can cause stress and negatively affect the quality of meat. This article aims to investigate the impact of pre-slaughter stress on meat quality. The literature suggests that pre-slaughter stress has a significant impact on meat quality, resulting in lower meat pH, increased drip loss, and decreased meat tenderness. When the body experiences stress, cortisol triggers the liver to release glucose into the bloodstream, which provides energy for the body to respond to the stressful situation. Both serum cortisol and blood glucose levels increased post-slaughter, with a post-slaughter mean value of 7.28 µg/dl for serum cortisol (compared to the initial mean value of 6.08 µg/dl), while the blood glucose values revealed a highly catabolic state, with mean post-slaughter values of 143.24 mg/dl compared to 129.11 mg/dl mean pre-slaughter results. The study highlights a direct relationship between increased serum cortisol levels and blood glucose values when comparing the pre- and post-slaughter values with the normal reference ranges, with a highly significant correlation between the mentioned parameters ($p<0.01$). The article also explores potential pre-slaughter indicators such as cortisol and blood glucose levels for predicting stress and meat quality in pigs. Improving pre-slaughter handling and reducing stress can lead to improved meat quality and economic benefits for the pork industry. In terms of pH24, a trust coefficient of 5.46–5.50 revealed the impact of pre-slaughter stress on meat pH, resulting in possible inferior meat in terms of quality. The study results showed a highly significant correlation between the cortisol and blood glucose values recorded post-slaughter and pH24 ($p<0.05$), emphasizing the impact of stress on meat quality and suggesting that both serum cortisol and blood glucose values can be used as specific indicators of meat pH and ultimately meat quality.

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**Keywords:** blood glucose; carcass evaluation; cortisol; pork; post-slaughter processing; stress.

## INTRODUCTION

The pork industry has been evolving to meet the demands of a growing global population. The quality of meat is a key factor in consumer satisfaction and can be influenced by various factors, including genetics, nutrition, and animal welfare (EFSA, 2022). Pre-slaughter stress is an important factor that can result in a physiological response that affects the meat quality parameters in pigs. During pre-slaughter, there are several risk factors that can result in stress; examples include feed and water deprivation (Dalla-Costa *et al.*, 2019), inadequate microclimatic conditions (Machado *et al.*, 2021), handling, transportation, and stunning (Rusu *et al.*, 2021). Therefore, understanding the impact of pre-slaughter stress on meat quality is important for maintaining consumer satisfaction and ensuring animal welfare.

Several studies have investigated the impact of pre-slaughter stress on meat quality in pigs. Peres *et al.* (2014) conducted a study on 36 pigs to assess the effect of low and high stress levels on carcass and meat quality. The results showed that pigs subjected to high levels of stress had significantly lower meat pH and increased drip loss compared to pigs subjected to low levels of stress. The study also found that pigs subjected to high levels of stress had significantly lower meat tenderness and higher shear force compared to pigs subjected to low levels of stress.

Stajkovic *et al.* (2017) conducted a review of 14 studies investigating the impact of pre-slaughter stress on pork quality. The review found that pre-slaughter stress has a significant impact on meat quality, resulting in lower meat pH, increased drip loss, and decreased meat tenderness. The review also highlighted that pre-slaughter stress can result in a darker meat colour and decreased water holding capacity.

Other studies (Sardi *et al.*, 2020a, b) investigated possible pre-slaughter indicators to predict stress and meat quality in heavy pigs. The study found that plasma cortisol concentration and heart rate variability were significantly associated with pre-slaughter stress and meat quality. The study also found that the percentage of muscle glycogen and pH were significantly associated with meat quality, suggesting that these indicators can be used to predict meat quality in heavy pigs. These indicators can be used to predict the impact of pre-slaughter stress on meat quality and identify pigs that are more susceptible to stress.

Peres *et al.* (2014) found that serum cortisol, blood lactate concentration, heart rate, and respiratory rate can be used to evaluate pre-slaughter stress in pigs. Their study also suggested that behavioural indicators, such as vocalization, can be used to identify pigs that are experiencing stress during transport and handling.

Other potential pre-slaughter indicators include the percentage of muscle glycogen, pH, and blood parameters such as glucose, creatinine kinase, and lactate dehydrogenase. These indicators can be used to assess

muscle energy metabolism and predict meat quality in pigs.

The aim of the current study was to investigate the impact of pre-slaughter stress on meat quality by exploring potential pre-slaughter indicators such as cortisol and blood glucose levels, to assist in the prediction of pre-slaughter stress and its influence on meat quality in pigs.

## MATERIALS AND METHODS

The study was conducted over a period of 6 months between June–December 2022 on 264 Duroc x Landrace crossbred pigs from a commercial pig farm in the north-eastern part of Romania, to investigate stress levels and the quality of the carcass in terms of pH values. Pigs (both males and females) aged 3 to 4 months old and weighing 80–110 kg were housed in 13.2 $m^2$ pens in an indoor finishing facility, on partially slatted floors, with a total surface area of 1.65 $m^2$/pig (8 pigs per pen). Pigs were provided feed and water ad libitum. The feed was calculated to provide at least 16% of crude protein with 0.7% lysine content or 21 g/day per individual. The inclusion criteria considered clinically healthy swine, without any obvious clinical pathology (no visible apathy, locomotory discomfort, diarrhoea, or respiratory symptoms) as such pathologies will lead to increased catabolic states with raised serum cortisol or blood glucose values in their acute forms. The pre-slaughter operations such as handling and transportation were performed on the same day as stunning, which was conducted at the slaughter plant. Transportation took 1 hour over a distance of 48 km, 0.3 km of which was on unpaved road and the remaining paved. The pigs were transported using a truck with free ventilation and a deck height of 120 cm. The inclination of the loading ramps was 15 degrees. The calculated density was 210 kg/$m^2$, providing 0.7 $m^2$/pig. The Thermal Humidity Index (THI) was 41, while the monthly THI for the entire period varied from 40 (December) to 64 (July).

Biochemistry was performed in an accredited laboratory to determine the serum cortisol and blood glucose levels. Samples were collected twice, with a 2-week interval between collections, both pre- and immediately post-slaughter. Blood samples were collected from the jugular vein in vacutainers containing clot activators using a sterile needle and syringe. The animals were handled with care to avoid causing excessive stress during the collection process. The collected blood samples were allowed to coagulate and then centrifuged to separate the serum from the blood cells. Serum cortisol concentrations were determined using a Demeditec solid phase ELISA kit (DEH 3388). Absorbance was measured using a Multiskan FC microtitre plate photometer (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm. The detectable range was between 10 and 750 ng/ml.

Post-slaughter processing and carcass evaluation were carried out in accordance with meat industry regulations. The pH was measured in the longissimus lumborum (LL) 45 min after stunning (pH45) and after 24 hours of

carcass chilling (pH24). These parameters (pH45 and pH24) were determined using a WTW 3310 pH meter and combination electrode (WTW-Wissenschaftlich-Technische Werkstaetten GmbH, Weilheim, Germany), calibrated with standard solutions of pH 4.01 and 7.00 at 20ºC.

The statistical interpretation was performed using MS Excel 2019, and using Fisher`s exact test and contingency tables to compare the pre- and post-slaughter values for each individual. The *P*-value representing statistical significance was set to $P<0.05$.

## RESULTS

Stress is known to have a significant impact on the meat quality of pigs, both in terms of physical characteristics and chemical composition. Pigs that experience stress prior to slaughter are more likely to have meat that is tough, dry, and less flavourful than those that are not stressed. Additionally, the colour of the meat may be affected, with stressed pigs producing meat that is lighter in colour than non-stressed pigs.

The aim of the current study was to investigate the effects of stress on glucose metabolism and cortisol levels and its impact on meat quality.

Both serum cortisol and blood glucose levels increased post-slaughter, with a mean post-slaughter value of 7.28 µg/dl for serum cortisol (compared to the initial mean value of 6.08 µg/dl), while the blood glucose values revealed a highly catabolic state, with mean post-slaughter values of 143.24 mg/dl compared to the pre-slaughter mean of 129.11 mg/dl.

The results also revealed a direct relationship between increased serum cortisol levels and blood glucose values when comparing the pre- and post-slaughter values with the normal reference ranges, with a highly significant correlation between the mentioned parameters ($p<0.01$), as shown in *Table 1*.

Cortisol is a hormone that is released by the adrenal glands in response to stress, and it plays a crucial role in regulating blood glucose levels. When the body experiences stress, cortisol triggers the liver to release glucose into the bloodstream, which provides energy to help the body respond to the stressful situation. As a result, cortisol levels increase in response to stress, and this is often used as a marker of stress in animal welfare research. This increase in cortisol is often accompanied by elevated blood glucose levels, as the liver releases more glucose into the bloodstream to provide energy for the body to deal with the stress.

The results presented in *Table 2* show the pH45 and pH24 values recorded in the current study, suggesting a decrease in pH value due to the impact of pre-slaughter stress.

Meat pH24 refers to the pH level of meat 24 hours after slaughter, which is an important indicator of meat quality. The ideal pH range for meat is between 5.5 and 5.8, which is slightly acidic. When the pH level of meat is too low, it can result in a dark, firm, and dry meat with a shorter shelf life. On the other hand, if the pH level is too high, it can lead to pale, soft, and exudative meat, which is also not desirable.

**Table 1 –** Serum cortisol and glucose levels before and after slaughter

| Result | Serum cortisol (µg/dl) | | Blood glucose (mg/dl) | |
|---|---|---|---|---|
| | Pre-slaughter | Post-slaughter | Pre-slaughter | Post-slaughter |
| Mean | 6.08 | 7.28 | 129.11 | 143.24 |
| Min. | 5.73 | 7.04 | 111 | 131 |
| Max. | 6.45 | 7.60 | 147 | 159 |
| CI mean | [6.03–6.11] | [7.25–7.32] | [127.12–131.15] | [141.76–147.11] |

**Table 2 –** Post-slaughter pH45 and pH24 values

| Parameter | pH45 | pH24 |
|---|---|---|
| Mean | 6.67 | 5.48 |
| Min. | 6.35 | 5.32 |
| Max. | 6.99 | 5.61 |
| CI mean | [6.63–6.72] | [5.46–5.50] |

In terms of pH24, a trust coefficient of 5.46–5.50 revealed the impact of pre-slaughter stress on meat pH, resulting in possible inferior meat in terms of quality according to meat classification standards based on pH24 (5.50–5.60).

There was also a highly significant correlation between the cortisol and blood glucose values recorded post-slaughter and pH24 ($p<0.05$), emphasizing the impact of stress on meat quality in pigs and showing that both serum cortisol and blood glucose values could be used as specific indicators for meat pH and ultimately meat quality.

## DISCUSSION

The pork industry generates billions of dollars annually, and the quality of pork meat is a critical factor that determines the industry's success. Pre-slaughter stress is a significant issue that can negatively impact meat quality in pigs. This study assessed the impact of pre-slaughter stress on pork meat quality and explored potential pre-slaughter indicators that can predict stress and meat quality in pigs.

During stress, the level of blood glucose tends to increase as part of the body's "fight or flight" response. This is because the body releases hormones such as adrenaline and cortisol, which stimulate the liver to release glucose into the bloodstream, providing energy to the muscles to deal with the stressful situation.

Our results in terms of cortisol values were similar to those reported in other studies (Tang *et al.*, 2018; Zeng *et al.*, 2019; Wang *et al.*, 2016), in which handling and transportation to the slaughtering plant, as pre-slaughter stress factors, were shown to have negative impacts on overall metabolic activity, highlighting an increase in serum cortisol levels due to stimulation of the hypothalamus-pituitary-adrenal axis. However, compared to previous studies (Li *et al.*, 2020; Zhang *et al.*, 2018), we found higher blood glucose values in the current study.

During stressful situations, cortisol levels increase due to activation of the hypothalamic-pituitary-adrenal (HPA) axis, this being a physiological response to stress. Cortisol stimulates the release

of glucose from the liver and adipose tissue, which increases blood glucose levels and provides energy for the body to cope with the stress (Bhatnagar *et al.*, 2012). In acute stress, cortisol levels rise rapidly and reach a peak within minutes to hours, leading to an initial increase in blood glucose levels, which may return to normal or decrease below normal levels as the stress resolves (Epel *et al.*, 2001).

However, if the stress is prolonged or chronic, the body's resources to produce glucose become depleted, leading to a decrease in blood glucose levels (Rosmond *et al.*, 2000). In addition, cortisol inhibits the uptake and utilization of glucose by the muscles and other tissues, leading to further hypoglycemia (Bhatnagar *et al.*, 2012).

In chronic stress, cortisol levels may remain elevated for prolonged periods, leading to a depletion of glucose stores and decreased glucose tolerance, which can lead to hypoglycemia and insulin resistance (Rosmond *et al.*, 2000). However, decreased insulin sensitivity and impaired glucose metabolism may lead to elevated blood glucose levels in the long term too, explaining the results presented in previous studies where the preparation for and duration of transportation exceeded 24 hours.

Pre-slaughter stress in pigs can also lead to decreased meat pH, which is an important factor that affects meat quality. The pH of meat reflects the level of acidity or alkalinity, which is related to the rate and extent of postmortem biochemical changes that occur in the muscle tissue. In general, the pH of meat is relatively stable immediately after slaughter, but it can decline over time due to the production of lactic acid by postmortem glycolysis.

The impact of pre-slaughter stress on meat quality can be attributed to several factors, but one of the primary factors is the depletion of muscle glycogen. Muscles store glycogen, which is converted to lactic acid during exercise or stress. During pre-slaughter handling and transport, pigs can experience glycogen depletion (Sardi, 2020a). One of the most significant changes is the decrease in muscle pH, which can affect water-holding capacity and meat colour. High levels of stress during pre-slaughter handling can result in a rapid decline in muscle pH post-mortem, leading to pale, soft, and exudative (PSE) meat. By causing changes in the biochemical composition of the meat, leading to altered pH, pre-slaughter stress will ultimately impact the quality of meat products. Stress can also result in the accumulation of metabolic waste products in muscle tissue, which can accelerate the postmortem degradation of the meat and reduce its shelf-life. These changes can negatively affect the sensory properties, nutritional value, and overall consumer acceptance of the meat product.

A study by Warris *et al.* (1994) found that pigs that were subjected to a stressful environment prior to slaughter had lower pH values, while the meat was significantly tougher and less juicy than in pigs that were not stressed. Similarly, a study by Mota-Rojas *et al.* (2007) found that pigs that were subjected to handling and transport stress prior to slaughter had meat that was less tender and less flavourful than pigs that were not stressed.

Unlike long-term pre-slaughter stress, such as fighting, cold weather, fasting and transit, which occurs 12 to 48 hours prior to slaughter and depletes muscle glycogen, resulting in meat that has a higher pH, darker colour, and is drier, short-term acute stress such as excitement or fighting immediately prior to slaughter produces lactic acid from the breakdown of glycogen (Malancus *et al.*, 2022), resulting in meat that has a lower pH, lighter colour, reduced water-binding capacity, and is possibly tougher.

The study thus proves that stress leads to an increase in the production of cortisol, a hormone that can break down muscle tissue leading to a decrease in meat quality as demonstrated by the study conducted by Barton-Gade (2001).

In order to reduce the impact of pre-slaughter conditions on meat quality in pigs, farmers can implement environmental and management strategies such as providing appropriate stocking densities, reducing noise levels, and minimizing handling frequency to mitigate pre-slaughter stress in pigs, ultimately improving animal welfare and product quality.

## CONCLUSIONS

Pre-slaughter stress has a significant impact on pork meat quality, resulting in lower meat pH, increased drip loss, and decreased meat tenderness. Potential pre-slaughter indicators, such as plasma cortisol concentration and blood glucose values, can be used to predict stress and meat quality in pigs. However, given the non-specific response of the body that stress involves it might be unwise to use a limited number of parameters as specific indicators for the quality of meat in pigs. Further studies on a larger number of individuals and parameters conducted on several farms may allow a broader interpretation of the impact of pre-slaughter stress in pigs. Nonetheless, both serum cortisol and blood glucose levels give an insight into stress-related biochemical changes and can help farmers take specific decisions to improve the welfare of the pig population. Implementing strategies to reduce pre-slaughter stress, such as minimizing handling and transport time and improving staff training, should improve meat quality and benefit the pork industry economically.

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